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Research Paper

Tat-HA-NR2B9c attenuate oxaliplatin-induced neuropathic pain

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ABSTRACT

Oxaliplatin is a commonly used chemotherapy drug, which can produce acute and chronic peripheral neurotoxicity. Currently, there is no good therapeutic drug in clinic. Excessive stimulation of *N*-methyl-D-aspartate receptors (NMDARs) is crucial for the transmission of pain signals. However, directly inhibiting NMDARs can cause severe side effects because they have key physiological functions in the Central nervous system (CNS). Several years ago, we prepared a polypeptide Tat-HA-NR2B9c which can disturb NMDARs–postsynaptic density protein-95 (PSD-95) interaction. In this study, we studied whether Tat-HA-NR2B9c could be an effective treatment for oxaliplatin-induced neuropathic pain. To conform it, a rat model of oxaliplatin-induced neuropathic was established, and analgesic effect of Tat-HA-NR2B9c was studied. Here, we show that oxaliplatin induces the interaction of NMDARs with PSD-95. Uncoupling the complex by Tat-HA-NR2B9c has potent analgesic effect in oxaliplatin-induced cold hyperalgesia and mechanical allodynia without suppressing general behavioral. Tat-HA-NR2B9c neither inhibits NMDARs function nor impacts antitumor activity of oxaliplatin. Thus, this new drug may serve as a treatment for oxaliplatin-induced neuropathic pain, perhaps without major side effects.

1. Introduction

Oxaliplatin is a third-generation platinum chemotherapy drug that is widely used in the treatment of many solid tumors including bowel cancer, lung cancer, ovarian cancer and pancreatic cancer [Wahlman et al., 2018]. However, about 74% patients receiving oxaliplatin suffer from acute symptoms and 48% suffer from persistent symptoms [Alejandro et al., 2013]. Currently, there is no effective treatment for neuropathic pain caused by chemotherapy, which is due in part to the difficulty in translating findings obtained from preclinical rodent models of chemotherapy induced peripheral neuropathy (CIPN) to clinic [Shidahara et al., 2016]. CIPN is a common neurological complication and major concern in oncology practice, given the lack of effective treatment and the increasing number of cancer survivors [Zhou et al., 2016]. Thus, finding therapeutic targets and developing effective analgesics have been considered very meaningful to clinic.

Oxaliplatin is metabolized to oxalate and dichloro (1, 2-diaminocyclohexane) platinum [Pt (dach) Cl_2] [Graham et al., 2000]. Oxaliplatin and oxalate may alter voltage-gated Na^+ channels and cause peripheral nerve injury, cold hyperalgesia and mechanical allodynia [Grolleau et al., 2001; Sakurai et al., 2009]. Central sensitization is a high reactivity of the spinal dorsal horn neurons to sensory input, which is a mechanism leading to chronic pain [Woolf, 1983]. Central sensitization is important in the development and maintenance of chronic pain. Long lasting harmful peripheral stimulation can activate NMDARs, trigger intracellular signaling cascade in the dorsal sensory neurons [Mihara et al., 2011; Qu et al., 2009]. Thus, blockade of NMDARs should be logically effective. Unfortunately, NMDARs play an important role in neural circuit rebuilding. NMDA receptor antagonists exhibit good analgesic effects in animal models, but clinical applications are limited because they have significant side effects [Chaplan et al., 1997; Mao et al., 1993; Ren and Dubner, 1993]. PSD-95 is a scaffolding protein, that binds both NMDARs 2B subunits (NR2B) and neuronal nitric oxide synthase (nNOS). The macromolecular signaling complex couples NMDAR activity to the production of NO (nitric oxide) [Aarts et al., 2002]. NO has been reported to induce NMDA-induced hyperalgesia [Kitto et al., 1992]. Targeting PSD-95 may be an ideal therapeutic approach for pain treatment. Recently, we constructed a

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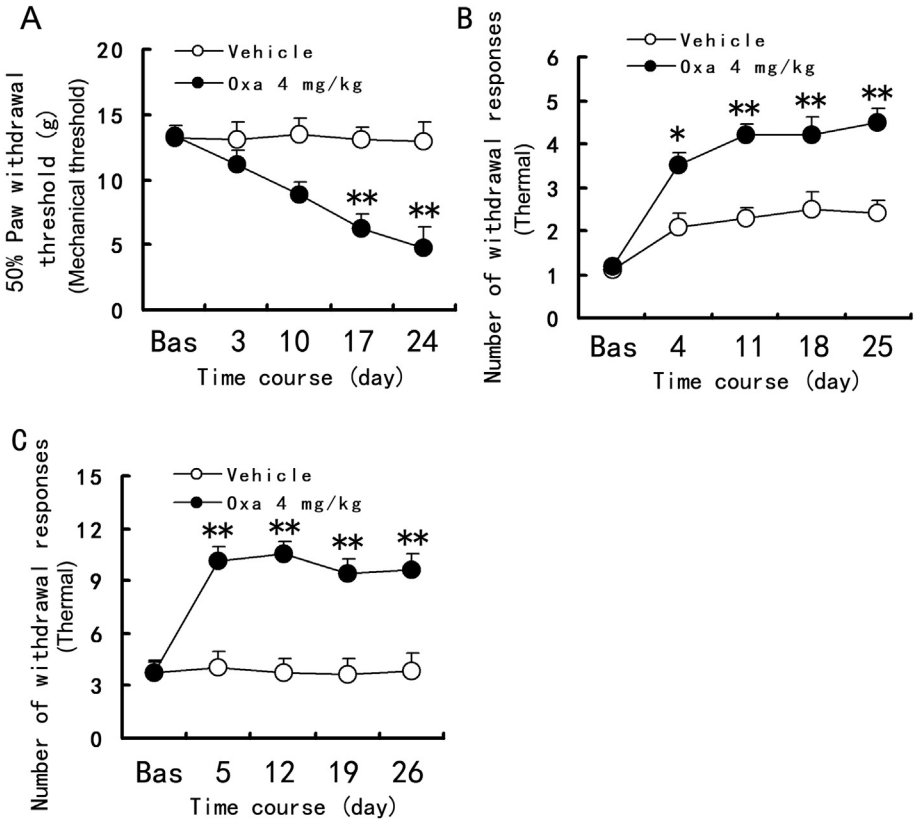

Fig. 1. Effects of oxaliplatin on mechanical allodynia (A), cold hyperalgesia (B, acetone test; C, cold plate test) in rats. Oxaliplatin (Oxa), Tat-HA-NR2B9c (9c). ($n = 10$ for vehicle, $n = 11$ for oxaliplatin). Data are mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle.

chimeric peptide Tat-HA-NR2B9c, containing the last 9 amino acids residues carboxyl of NR2B COOH-terminal, an influenza virus hemagglutinin epitope-tag and 11-mer Tat protein transduction domain. The chimeric peptide showed significant brain protection effect [Zhou et al., 2012; Zhou et al., 2015]. Here we show that NMDAR–PSD-95 interaction modulates oxaliplatin-induced pain. Dissociating NMDAR–PSD-95 coupling significantly attenuated oxaliplatin-induced neuropathic pain. Thus, we identify a novel approach to treat oxaliplatin-induced pain.

2. Materials and methods

2.1. Animals

All methods involved in animal were approved by the Institutional Animal Care and Use Committee of Nanjing University (Approval No., GY20160108), and all procedures involving animals were carried out in accordance with National Institute of Health guidelines for laboratory animals. Adult male Sprague-Dawley rats (220–250 g) were purchased from B&K Universal Group Limited, Shanghai. And 6-week-old male BALB/c mice (20–25 g) were purchased from Nanjing University Animal Center. All animals were housed at 22 ± 2°Con a 12 h light/dark cycle with free access to water and food.

2.2. Experimental design

Adult male Sprague-Dawley rats were randomized into four experimental groups: vehicle, oxaliplatin, oxaliplatin + Tat-HA-NR2B9c (50 ng), oxaliplatin + Tat-HA-NR2B9c (100 ng). Oxaliplatin was (Sigma-Aldrich, St Louis, Missouri, USA) dissolved in 5% glucose solution. In sham and oxaliplatin groups, 5% glucose solution or oxaliplatin (4 mg/kg) was, respectively, injected intraperitoneally (i.p.) twice a week for 4 weeks (on days 1, 2, 8, 9, 15, 16, 22, and 23). In Tat-HA-NR2B9c-treated groups, in addition to oxaliplatin injection, Tat-HA-NR2B9c was administered intrathecal injection on day 24, 25 and 26. Mechanical hyperalgesia measurement, acetone test and cold-plate test were measured on 24 days, 25 days and 26 days respectively.

2.3. Drugs

Tat-HA-NR2B9c was prepared in our laboratory [Zhou et al., 2012]. Oxaliplatin (Sigma-Aldrich, St Louis, Missouri, USA) was dissolved in 5% dextrose (1 mg/ml) and prepared fresh for daily use. Oxaliplatin was administered 4 mg/kg i.p. twice a week for 4 weeks. This kind of administration was proved safety [Cavaletti et al., 2001].

2.4. Intrathecal injection of Tat-HA-NR2B9c

The intrathecal injection was administered according to the procedure described previously with slight modification [Zhou et al., 2016]. Simply, rats were artificially restrained to maintain the position of the needle and then a 25-μl Hamilton syringe with a 30-gauge needle was used for intrathecal (i.t.) injection into the L5–6 interspace, eliciting a tail-flick. After injection, the syringe was held for a few seconds.

2.5. Mechanical hyperalgesia measurement

Mechanical hyperalgesia was measured by von Frey test [Mihara et al., 2011]. Each rat was placed in a transparent plastic box with a wire mesh and allowed them to get used to it for 30 min before testing. Von Frey filaments of varying forces (2–15 g) were applied serially to the paw using the up-down method. The 15 g filament was selected as the upper limit cutoff for testing. A positive response was noted if the paw was sharply withdrawn.

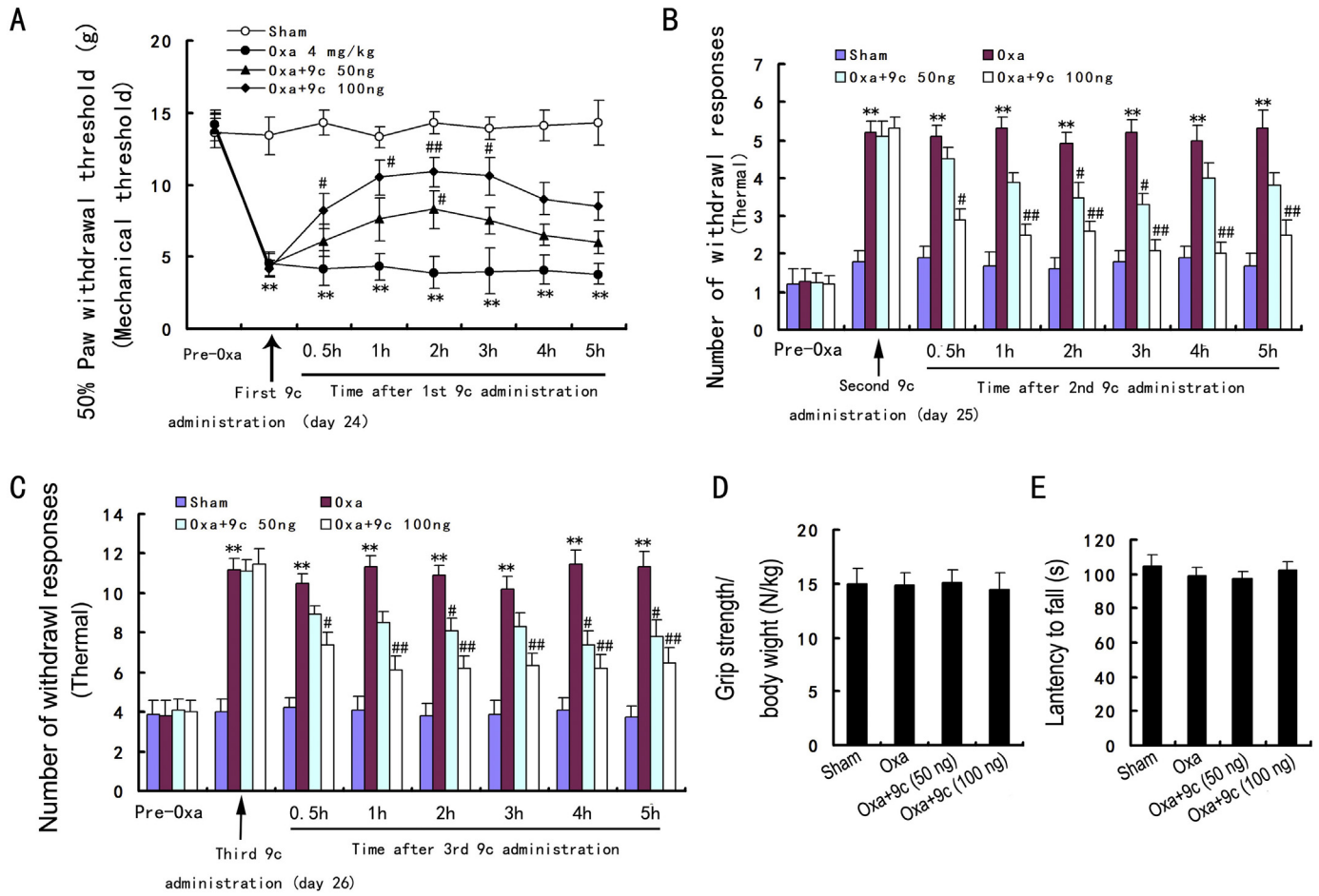

Fig. 2. Tat-HA-NR2B9c attenuated oxaliplatin-induced mechanical and cold hypersensitivity. (A) mechanical allodynia, (B-C) cold hyperalgesia (B, acetone test; C, cold plate test) in rats after oxaliplatin-treatment, (D) grip strength tests, (E) rota-rod. Oxaliplatin (Oxa), Tat-HA-NR2B9c (9c). ($n = 10$ for sham, $n = 11$ for oxaliplatin, n = 10 for 9c 50 ng and n = 11for 9c 100 ng). Data are mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. sham; #P < 0.05, ##P < 0.01, vs. oxaliplatin.

2.6. Cold plate test and acetone test for cold hyperalgesia

Cold hyperalgesia was assessed at 4 °C by a Cold/Hot Plate as described previously [Cheng et al., 2017]. It was performed as the number of foot withdrawal responses in 1 min after cold stimulus. The testing was repeated 3 times with approximately 5 min interval. Cold hyperalgesia was also measured by acetone test, rats were placed in a clear plastic box (20 cm × 17 cm × 13 cm) and got used for 30 min before testing. Fifty microliters of acetone was sprayed onto the plantar skin of both hind paws, and the number of withdrawal response was counted for 40 s. The results were reported as the average of 3 readings.

2.7. Grip strength test and Grip strength test

Rota-rod test for motor coordination. Rats were placed on a rotating rod and the latency to falling was measured for up to 2 min according to the method described.

previously [Sakurai et al., 2009]. The test was performed 3 times, and the rotating speed was 10 rpm.

2.8. Grip strength test for motor strength

This test was performed using tension gauge according to the method described previously [Sakurai et al., 2009]. Each rat was placed with both forepaws inside the front grip, and the strain gauge was zeroed. When the rat gripped the grid, it was steadily pulled backwards by the tail until its grip was broken. The reading on the strain gauge was recorded. The test was performed 4 times.

2.9. Tumor cytotoxicity assay

Male BALB/c mice (20–25 g) were injected CT26 (2×10^6) cells into the back as described previously [Cheng et al., 2017]. Murine CT26 (Colon Tumor #26) cells were developed in 1975 by exposing BALB/c mice to N-nitroso-*N*-methylurethane (NMU), resulting in a rapid-growing grade IV carcinoma that is easily implanted and readily metastasizes [Griswold and Corbett, 1975]. When the tumors grew to about 200–300 mm^3 size, mice were randomly assigned. Vehicle was treated with 5% dextrose (0.2 ml) for 2 weeks, and oxaliplatin group was treated with intraperitoneal oxaliplatin (10 mg/kg/week, 2 weeks, i.p.). Tat-HA-NR2B9c groups were administrated oxaliplatin (10 mg/kg/week, 2 weeks, i.p.), and Tat-HA-NR2B9c (50 and 100 ng/d, respectively, i.t. daily for 14 days). Tumor volume was measured with a caliper rule every five days, and was calculated as follows: TV (mm^3) D ($L \times W^2$)/2, L is length and W the shortest radius of the tumor (mm). Tumor weight was measured finally.

2.10. Coimmunoprecipitation

Lysis and coimmunoprecipitation of spinal cord dorsal horn was performed as described previously [Zhou et al., 2010]. Spinal cord dorsal horn lysate were lysed in buffer (50 mM Tris-HCl, 150 mM NaCl, 1% PMSF, 1 mM EDTA-Na, 0.1% SDS, 1% NP-40, 0.5% sodium deoxycholate, 0.02% sodium azide, 0.5‰ pepstatin A, 1‰ aprotinin,

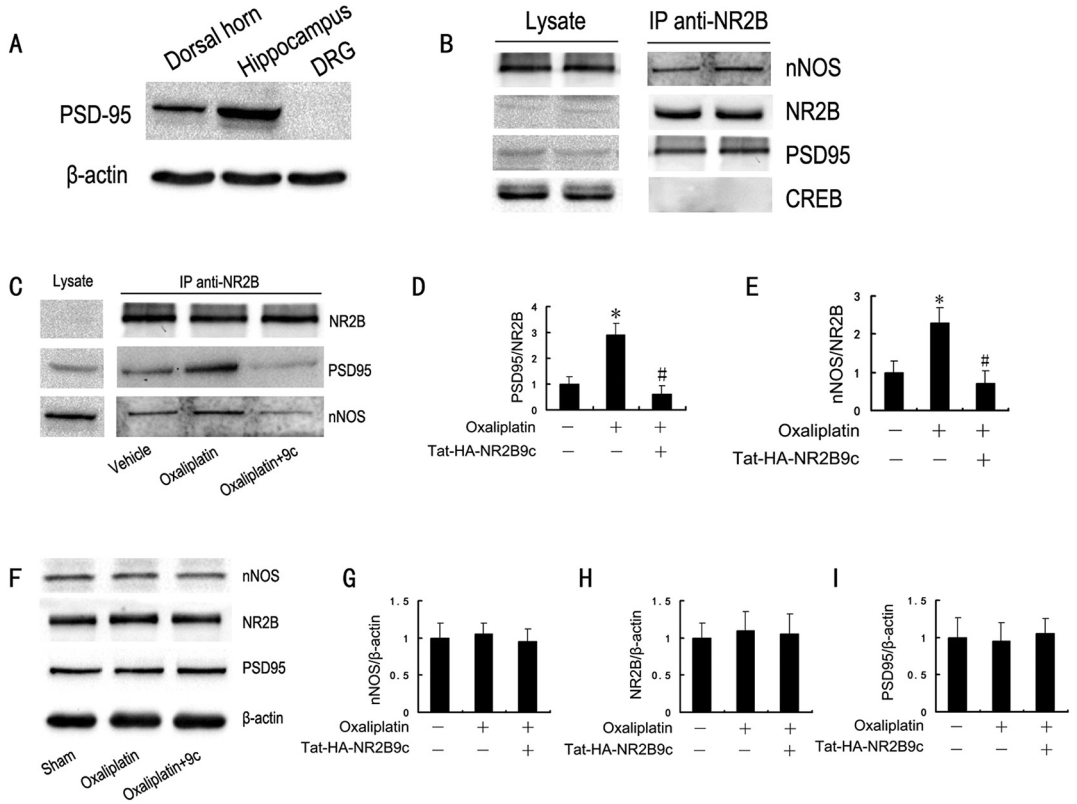

Fig. 3. Oxaliplatin increased PSD95 binding to NR2B in dorsal horn neurons. (A) PSD-95 expression in spinal dorsal horn, hippocampus and DRG. (B) Protein expression of normal spinal dorsal horn lysates and coimmunoprecipitates from spinal dorsal horn lysate using an antibody against NR2B. (C-E) Western immunoblots showing immunoprecipitates from the dorsal horn of rats preconditioned with Tat-HA-NR2B9c (100 ng), 30 min before being sacrificed (right lanes). Normal dorsal horn lysates with no immunoprecipitation (left lanes). (F-I) Western immunoblots showing protein expression before and after oxaliplatin treatment in dorsal horn neurons. ($n = 5$ for all groups). Data are mean ± SEM. (*$P < 0.05$. vs sham; #P < 0.05 vs oxaliplatin).

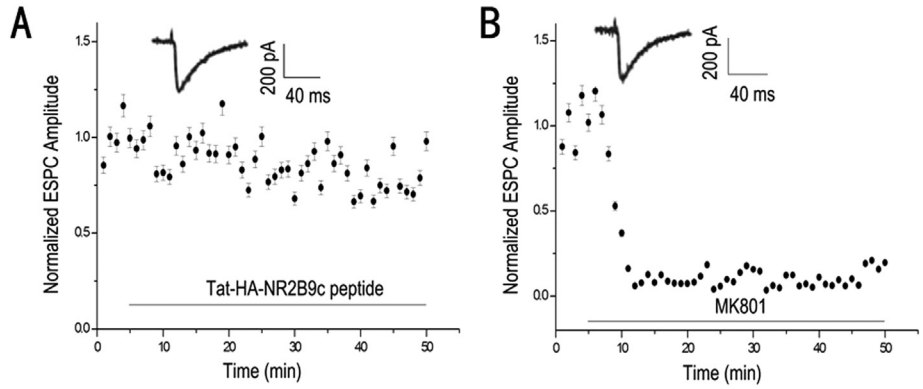

Fig. 4. Neurophysiological effects of Tat-HA-NR2B9c. (A) Effect of Tat-HA-NR2B9c on field EPSCs of dorsal horn neuron in spine slices. (B) Effect of NMDA on field EPSCs of dorsal horn neuron in spine slices.

and1‰ leupeptin.). The lysates were centrifuged at 12,000 × *g* for 15 min at 4 °C. The supernatant was preincubated at 4 °Cfor 1 h with 0.025 ml of protein G-Sepharose beads (Sigma-Aldrich), and then centrifuged to remove proteins that adhered nonspecifically to the beads and to obtain the target supernatant for the following IP experiment. Protein G Sepharose beads were incubated with rabbit anti-NR2B (1:200; Chemicon) for 3–4 h. The antibody-conjugated protein G-Sepharose beads and the target supernatant were added for incubation overnight at 4 °C. Immune complexes were isolated by centrifugation, washed four times with 0.05 M HEPES buffer, pH 7.1, containing 0.15 M NaCl, 0.15% Triton X-100, and 0.1×10^{-3} M sodium orthovanadate; and bound proteins were eluted by heating at 100 °C in loading buffer. Proteins were analyzed by immunoblotting.

2.11. Electrophysiological recordings of dorsal horn neurons

Spinal Cord slices were prepared from male SD rats as described previously [Xie et al., 2016]. Briefly, 3 weeks old rat was anesthetized with ethyl ether and decapitated, and the lumbar spinal cord at the L3-L6 level was removed through laminectomy. The spinal tissues were immediately placed in an ice-cold sucrose artificial cerebrospinal fluid (ACSF). The ACSF contain the following (in mM) 126 NaCl, 26 $NaHCO_3$, 20 glucose, 2.5 KCl, 1.25 $KHPO_4$, 1 $MgCl_2$, and 1 $CaCl_2$. ACSF was bubbled continuously with carbogen (95%O_2 and 5%CO_2) to adjust the pH to 7.4. Fresh slices were incubated in chamber with carbogenated ACSF and recovered at 34 °C for at least 1.5 h before they were transferred to recording chamber. The dorsal horn neurons were viewed

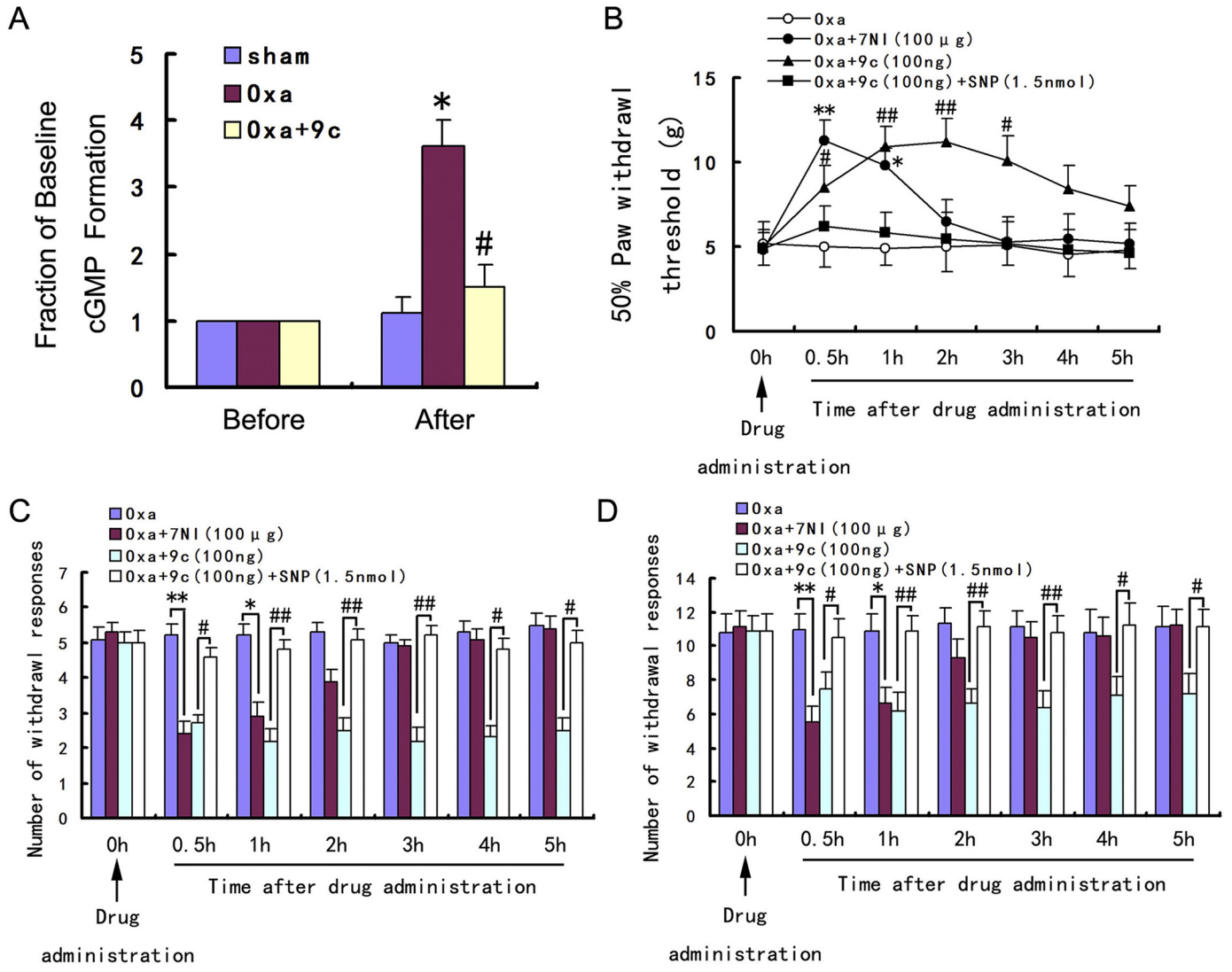

Fig. 5. Effects of Tat-HA-NR2B9c on cGMP production in dorsal horn neurons (A). 7-NI and SNP on oxaliplatin-induced pain in mechanical allodynia (B), cold hyperalgesia (C, acetone test; D, cold plate test). Oxaliplatin (4 mg/kg) was administered i.p. twice a week for 4 weeks. We carried out the drug evaluation on day 24, 25 and 26. 7-NI (100 μg/body) and SNP (1.5 nmol/body) were administered intrathecally. (A) cGMP production ($n = 5$). (B) The von Frey test. (C) Acetone test. (D) Cold plate test. ($n = 10$ for all groups). Data are mean ± SEM. *$P < 0.05$, vs. sham, #P < 0.05, vs. oxaliplatin; *P < 0.05, **$P < 0.01$, vs. oxaliplatin, #P < 0.05, ##P < 0.01, vs. SNP.

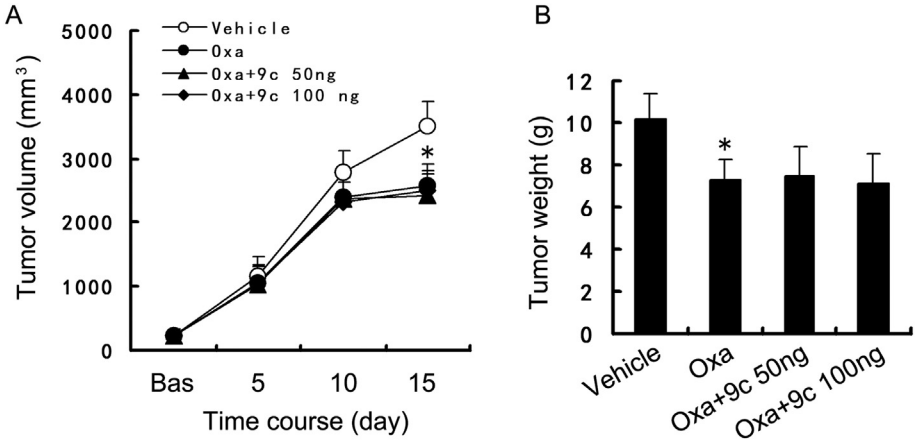

Fig. 6. Effect of Tat-HA-NR2B9c on the anti-tumor effect of oxaliplatin. (A) Effect of Tat-HA-NR2B9c on tumor volumes. (B) Effect of Tat-HA-NR2B9c on tumor weight. (n = 10 for all groups). Data are mean ± SEM. *P < 0.05 vs. vehicle.

under upright microscopy. The recording chamber was perfused at a rate of 4 ml min^{-1}, with an external recording solution that contained the following (in mM): 119 NaCl, 26 $NaHCO_3$, 25 glucose, 4 $CaCl_2$, 2.5 KCl, 1.3 $MgCl_2$, and1 NaH_2PO_4, bubbled with 95% O_2 and 5% CO_2 (300–310 mOsm). Excitatory postsynaptic responses of dorsal horn neurons were evoked through a constant-current pulse delivered by a bipolar tungsten electrode and recorded with Axonpatch-700B amplifier (Axon Instruments). The baseline was recorded at least 10 min to ensure the stability of the response. Tat-peptides or NMDA were applied in ACSF and recordings were continued for 50 min thereafter. Data were collected with pClamp 9.2 software and analyzed using Clampfit 9.2 (Molecular Devices).

2.12. Data analysis

Statistical significances between groups were analyzed by Student *t*-test. Comparisons among multiple groups were performed using one-way analysis of variance (ANOVA) (one factor) or two-way ANOVA (two factors), followed by Scheffe's post-hoc test. Comparisons between two groups were performed using a two-tailed Student's t-test. Data were presented as the mean ± SEM and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Oxaliplatin induced significant mechanical allodynia and cold hyperalgesia

Mechanical allodynia induced by oxaliplatin was performed in the von Frey test, and cold hyperalgesia was performed in acetone and cold-plate tests. Oxaliplatin was administered on days 1, 2, 8, 9, 15, 16, 22 and 23 twice a week for 4 weeks. And the cumulative dose of oxaliplatin is 32 mg/kg. Baseline values for all tests were measured before the first administration. The von Frey test was performed on day 0, 3, 10, 17 and 24. The acetone test was performed on Day 0, 4, 11, 18 and 25 and cold-plate test was performed on days 0, 5, 12, 19 and 26. Oxaliplatin significantly decreased the paw withdrawal thresholds (PWTs) on days 17 and 24 [Fig. 1A]. Oxaliplatin significantly increased the number of withdrawal response on days 4, 11, 18 and 25 in acetone test and increased the number of withdrawal response on days 5, 12, 19 and 26 in cold-plate test [Fig. 1B–C].

3.2. Tat-HA-NR2B9c attenuated oxaliplatin-induced mechanical and cold hypersensitivity

The pharmacodynamics of Tat-HA-NR2B9c on PWTs test, acetone test and cold plate test were measured on day 24, day 25 and day 26 respectively. Rats were treateded with Tat-HA-NR2B9c (50 ng) or Tat-HA-NR2B9c (100 ng) single time before PWTs test, acetone test and cold plate test respectively. Tat-HA-NR2B9c (100 ng) significantly increased the PWTs from 0.5–3 h after treatment of Tat-HA-NR2B9c [Fig. 2A]. Tat-HA-NR2B9c (100 ng) significantly decreased the number of responses in acetone and cold plate test from 0.5–5 h [Fig. 2B-C]. We also study whether the antihyperalgesic effects we observed for Tat-HA-NR2B9c were because of general behavioral suppression within the dose range. No significant difference was observed in all groups [Fig. 2D-E]. So the antihyperalgesic effects observed were not because of general behavioral suppression.

3.3. Oxaliplatin induced NR2B–PSD-95 association in the dorsal horn

Above data suggest that disrupting the NR2B–PSD-95 association benefit oxaliplatin induced pain. We wondered whether oxaliplatin increased NR2B–PSD-95 association. To address this hypothesis, first, we measured the protein expression of PSD95 in the hippocampal and spinal dorsal horn. It was only found in dorsal horn sensory neurons and not present in dorsal root ganglion [Fig. 3A]. Then, we performed coimmunoprecipitation experiments with the spinal dorsal horn. PSD-95 and nNOS were coimmunoprecipitated by anti-NR2B antibody [Fig. 3B]. Normal spinal lysate was positive controls, and nuclear protein cAMP-response element binding protein (CREB) was negative control. CREB was only founded in normal spinal tissue lysates [Fig. 3B].

Spinal NR2B-containing NMDA receptors were reported to be involved in oxaliplatin-induced mechanical allodynia in rats [Mihara et al., 2011]. Nitric oxide that generated from nitric oxide synthase contributes to central sensitization [Cheng et al., 2017]. We thus hypothesized that spinal NR2B–PSD-95 interaction may take part in the oxaliplatin-induced pain. To address this hypothesis, we tested the effect of Tat-HA-NR2B9c, a drug that selectively blocks NR2B–PSD-95 binding by immunoprecipitation. In another experiment, rats were pretreated with Tat-HA-NR2B9c (100 ng) (it), 30 min before collection of lumbar dorsal horn tissue. As expected, oxaliplatin significantly increased NR2B–PSD-95-nNOS complex in the dorsal horn of spinal cord [Fig. 3C-E]. Tat-HA-NR2B9c significantly decreased the complex [Fig. 3C-E]. The increase in the NR2B–PSD-95-nNOS complex was not due to protein overexpression because oxaliplatin did not change PSD95 and nNOS expression [Fig. 3F–I].

3.4. Tat-HA-NR2B9c did not inhibit NMDARs EPSCs

NMDAR dysfunction is implicated in multiple pain disorders include CIPN [Qu et al., 2009; Shirahama et al., 2012]. NMDAR antagonists exhibit powerful analgesic effects, but they also produce unacceptable severe side effects, including cognitive problems, hallucinations and even coma and are not routinely used for these purposes [Kelley et al., 2009; Kemp and McKernan, 2002; Koroshetz and Moskowitz, 1996]. Since the functional differences between blockade of NMDAR and disrupting NMDAR-PSD95 are not clear, we examined NMDAR currents. Bath application of Tat-HA-NR2B9c to spine slices does not inhibit NMDARs excitatory postsynaptic currents (EPSCs), while NMDA inhibit NMDARs EPSCs [Fig. 4A-B].

3.5. Tat-HA-NR2B9c played analgesic effect through NR2B–PSD-95-nNOS pathway in spine cord

We next studied whether Tat-HA-NR2B9c affected NMDAR downstream activation. cGMP (cyclic guanosine monophosphate) level was measured as surrogate measure of NO production by nNOS [Aarts et al., 2002]. Oxaliplatin significantly increased cGMP level that was suppressed by Tat-HA-NR2B9c [Fig. 5A]. We then employed nNOS selective inhibitor 7-NI (7-nitroindazole) and nitric oxide donor SNP (sodium nitroprusside) to study the exact mechanism. On day 24 (next day of last oxaliplatin treatment), acute administration of 7-NI (100 μg, i.t.) significantly restrained the reduction of PWT and cold pain induced by oxaliplatin, while SNP (1.5 nmol, i.t.) abolished the analgesic effect of Tat-HA-NR2B9c [Fig. 5B–D]. These data suggest a significant implication of that Tat-HA-NR2B9c played analgesic effect through NR2B–PSD-95-nNOS pathway in spine cord.

3.6. Tat-HA-NR2B9c did not affect the anti-tumor effect of oxaliplatin

In this research, we also investigated whether Tat-HA-NR2B9c affect the antitumor activity of oxaliplatin. Male BALB/c mice bearing CT26 tumors were employed. Oxaliplatin significantly inhibited the increase of tumor volumes. Tat-HA-NR2B9c did not attenuate tumor growth as compared to oxaliplatin alone [Fig. 6]. These preliminary results suggested that Tat-HA-NR2B9c did not attenuate anti-tumor activity of oxaliplatin.

4. Discussion

Oxaliplatin often causes severe peripheral neuropathy toxicity. The pain symptom can greatly diminish quality of life; create or worsen depression, insomnia, fatigue, and other symptom clusters [Hwang et al., 2016]. Although many efforts have been made, the underlying mechanism is not well understood and no effective therapy strategy available. Clinics are in urgent need for effective drugs. Our work proposed preventive effects of Tat-HA-NR2B9c on oxaliplatin-induced neurotoxicity in animal model. We hope that this finding may provide beneficial choice for the neuropathy treatment.

In recent years, several hypotheses have been proposed to explain the neurotoxicity of oxaliplatin. Oxaliplatin makes sensory neurons hyperexcitable and eventually generate spontaneous ectopic discharges [Adelsberger et al., 2000]. Oxaliplatin can also form adducts with mitochondrial DNA producing inhibition of replication, disruption of transcription and morphological abnormalities within mitochondria in DRG neurons, leading to a gradual energy failure [Canta et al., 2015]. The presence of abundant fenestrated capillary network and the absence of blood-brain barrier in DRG allow platinum drugs to preferentially accumulate in this region with easy access to sensory neurons [McWhinney et al., 2009]. Both NR2B and PSD-95 are distributed in dorsal horn especially in superficial laminae, making this area more receptive to pain [Nagy et al., 2004]. NMDAR dysfunction is implicated in multiple pain disorders including CIPN [Mihara et al., 2011; Qu et al., 2009]. NMDAR antagonists exhibit powerful analgesic effects, but since they produce unacceptable severe side effects, they are not routinely used for these purposes [Kelley et al., 2009; Kemp and McKernan, 2002]. Selective nNOS inhibitor can completely reverse oxaliplatin-induced mechanical allodynia [Mihara et al., 2011]. The inhibition of nNOS produces aggressive behavior [Nelson et al., 1995] and impairs learning and memory [Shirahama et al., 2012]. NMDAR activity is unaffected by genetically deleting PSD-95 in vivo [Migaud et al., 1998] or by suppressing its expression in vitro [Sattler et al., 1999]. Disrupting the NR2B–PSD-95 interaction may have better effects on neuropathic pain than directly blocking NMDAR. In order to investigate the hypothesis, we designed oxaliplatin-induced peripheral neuropathy model using SD rats. The rat model employed in this study was appropriate for mimicking human symptoms caused by oxaliplatin [Ushio et al., 2012]. We showed that binding of PSD-95 to spinal NR2B is important for oxaliplatin-induced neuropathic pain. Perturbing such interactions with the chimeric polypeptide, Tat-HA-NR2B9c, reduced cold hyperalgesia and mechanical allodynia. Tat-HA-NR2B9c did not impact the performance of rats on the rota-rod and grip strength tests. Conventional NMDAR antagonists MK801 and ketamine produced motor dysfunction [Chaplan et al., 1997]. Tat-HA-NR2B9c did not inhibit NMDARs EPSCs, while MK801 inhibit NMDARs EPSCs. These findings may explain why Tat-HA-NR2B9c had few side effects. The analgesic effects began 30 min after drug administration, and lasted for the full 5-h testing period. We found that higher dose (100 ng) showed better behavioral efficacy; therefore, we used this higher dose in subsequent experiments. This analgesic effect could be abolished by NO donor. What's more, acute administration of a nNOS inhititor 7-NI significantly inhibited the oxaliplatin-induced pain. Oxaliplatin significantly increased cGMP level that was suppressed by Tat-HA-NR2B9c.

Our findings suggest that coupling between spine NR2B and PSD95 is crucial for oxaliplatin-induced neuropathic pain. Targeting interaction downstream of NMDARs without receptor blockade could avoid the neurological side effects associated with NMDARs antagonists. Thus, Tat-HA-NR2B9c may serve as a novel analgesic for oxaliplatin-induced neuropathic pain.

Author contributions

Conceived of or designed study: H.-H.Z, X.-P.Q, Y.-J.Z and W.-H.G; Performed research: H.-H.Z, L.Z, H.-X.Z and B.-R.X; Analyzed data: J.-P.Z, X.-P.Q and Y.-J.Z; Wrote the paper: H.-H.Z.

Conflicts of interest

None.

Acknowledgments

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