American Pain Society
RESEARCH
EDUCATION
TREATMENT
ADVOCACY
PUBLISHED BY ELSEVIER

The Journal of Pain, Vol 14, No 2 (February), 2013: pp 205-214
Available online at www.jpain.org and www.sciencedirect.com

Spinal Astrocyte Gap Junctions Contribute to Oxaliplatin-Induced Mechanical Hypersensitivity

Seo-Yeon Yoon,* Caleb R. Robinson,† Haijun Zhang,† and Patrick M. Dougherty†

**Laboratory of Molecular Signal Transduction, Center for Neural Science, Korea Institute of Science and Technology, Seoul, South Korea.*

†Department of Anesthesia and Pain Medicine Research, The University of Texas M.D. Anderson Cancer Center, Houston, Texas.

Abstract: Spinal glial cells contribute to the development of many types of inflammatory and neuropathic pain. Here the contribution of spinal astrocytes and astrocyte gap junctions to oxaliplatin-induced mechanical hypersensitivity was explored. The expression of glial fibrillary acidic protein (GFAP) in spinal dorsal horn was significantly increased at day 7 but recovered at day 14 after oxaliplatin treatment, suggesting a transient activation of spinal astrocytes by chemotherapy. Astrocyte-specific gap junction protein connexin 43 (Cx43) was significantly increased in dorsal horn at both day 7 and day 14 following chemotherapy, but neuronal (connexin 36 [Cx36]) and oligodendrocyte (connexin 32 [Cx32]) gap junction proteins did not show any change. Blockade of astrocyte gap junction with carbenoxolone (CBX) prevented oxaliplatin-induced mechanical hypersensitivity in a dose-dependent manner and the increase of spinal GFAP expression, but had no effect once the mechanical hypersensitivity induced by oxaliplatin had fully developed. These results suggest that oxaliplatin chemotherapy induces the activation of spinal astrocytes and this is accompanied by increased expression of astrocyte-astrocyte gap junction connections via Cx43. These alterations in spinal astrocytes appear to contribute to the induction but not the maintenance of oxaliplatin-induced mechanical hypersensitivity. Combined, these results suggest that targeting spinal astrocyte/astrocyte-specific gap junction could be a new therapeutic strategy to prevent oxaliplatin-induced neuropathy.

***Perspective:** Spinal astrocytes but not microglia were recently shown to be recruited in paclitaxel-related chemoneuropathy. Here, spinal astrocyte gap junctions are shown to play an important role in the induction of oxaliplatin neuropathy.*

***Key words:** Chemotherapy, connexin, GFAP, carbenoxolone.*

Peripheral neuropathy is one of the most common side effects following chemotherapy and causes prominent sensory disability and persistent pain even after chemotherapy has been discontinued.[19,29,41,55] Oxaliplatin is the third-generation platinum-based compound used as the primary therapy for metastatic colorectal cancer and other malignancies such as lung, breast, and ovarian cancers.[20,23,44,50] Oxaliplatin induces prominent neuropathic pain that is characterized by pronounced cold and mechanical hypersensitivity and spontaneous pain.[4,6,52] Several types of neuroprotective compounds including thiols, neurotrophic factors, anticonvulsants, and antioxidants have been tested in preventing oxaliplatin-induced neuropathy, but no definite effects have been found in clinical studies.[3] Thus, it still remains a high priority to identify safe and effective approaches to prevent or ameliorate oxaliplatin-induced painful neuropathy.

Spinal astrocytes have been shown to contribute to the development of chronic pain in various conditions including surgery, inflammation, and nerve injury.[9,22,26,28,39] Astrocytes are typically interconnected by gap junctions to form the organization of a functional syncytium.[12] Gap junctions are formed by the linking of 2 hemichannels; 1 in each of 2 opposing cells, with each hemichannel composed of a hexamer of gap junction proteins called connexins (Cx), the most common of which in astrocyte hemichannels is connexin 43 (Cx43).[35] Gap junctions

Received August 23, 2012; Revised October 29, 2012; Accepted November 7, 2012.
This work was supported by National Institutes of Health grant NS46606 and National Cancer Institute grant CA124787.
The authors declare no conflicts of interest.
Address reprint requests to Patrick M. Dougherty, Department of Pain Medicine, Unit 409, The University of Texas M.D. Anderson Cancer Center, 1400 Holcombe Blvd, Houston, TX 77030. E-mail: pdougherty@mdanderson.org
1526-5900/$36.00

http://dx.doi.org/10.1016/j.jpain.2012.11.002

appear to play an important role in the expression of various types of inflammatory and neuropathic pain. Spinal astrocyte gap junction protein is increased following chronic constriction of sciatic nerve[57]; and blockade of gap junctions reduces peripheral nerve injury-induced central sensitization in medullary dorsal horn.[13,49] We have previously shown that paclitaxel-induced painful neuropathy is associated with the activation of spinal astrocytes.[61] It is not known, however, whether spinal astrocytes are also activated in oxaliplatin-induced neuropathy and whether astrocyte involvement in chemotherapy-induced neuropathy may engage a gap-junction mechanism. In the present study, we examined the activation of spinal astrocytes and the expression of the astrocyte-specific gap junction protein as well as gap junction proteins in neurons and oligodendrocytes in a rat model of oxaliplatin-induced peripheral neuropathy. In addition, we tested the effect of carbenoxolone (CBX) (Sigma-Aldrich Corp, St. Louis, MO), a gap junction decoupler, on mechanical hypersensitivity and spinal astrocyte activation during and after oxaliplatin treatment.

Methods

Animals

The experiments were performed using 200 male Sprague-Dawley rats (330–380 g; Harlan Laboratories, Indianapolis, IN) housed in colony cages with free access to food and water and maintained in temperature- and light-controlled rooms (23 ± 2°C, 12/12-hour light/dark cycle with lights on at 0700) for at least 1 week prior to the study. One hundred thirty animals were used in the Western blot and immunohistochemistry studies (4–5 per group), and 70 animals were used in the behavioral pharmacology studies (6–8 per group). The specific allotment of animals to each experiment is detailed below. The experimental protocols for animal usage were reviewed and approved by the Institutional Animal Care and Use Committee of The University of Texas M.D. Anderson Cancer Center and conformed to National Institutes of Health guidelines (NIH publication No. 86-23, revised 1985).

Drug Administration

Oxaliplatin (Tocris Bioscience, Bristol, United Kingdom) was prepared by diluting to 1 mg/mL in saline (.9%) from a stock solution (5 mg/mL in 5% dextrose) and injected intraperitoneally at a dosage of 2 mg/kg every other day for a total of 4 injections (days 1, 3, 5, and 7).[7] Control animals received an equivalent volume of vehicle, which consisted of 5% dextrose and saline in the same final concentration as the oxaliplatin solution.

CBX was diluted in saline. For assessment of its preventive effect on oxaliplatin-induced neuropathic pain, CBX (10 μL per injection) was injected intrathecally by lumbar puncture at sacral level, starting 24 hours prior to the first dose of oxaliplatin (day 0) and continued once daily for 7 consecutive days for a total of 8 injections (days 0–7). On days when both oxaliplatin and CBX were to be administered (days 1, 3, 5, and 7), CBX was given 30 minutes prior to oxaliplatin. For assessment of the effect of CBX on established oxaliplatin-induced neuropathic pain, CBX was administered once daily on days 14 to 21 after chemotherapy. Doses of carbenoxolone ranged from 1 to 25 μg per animal per day. Control animals received 10 μL saline in the same fashion.

Behavioral Assessments

Mechanical withdrawal threshold was determined for all rats using an ascending series of von Frey filaments as previously described.[10,59] Rats were placed in a clear plexiglas compartment (25.4 × 25.4 × 10.16 cm; 10 × 10 × 4 in) on an elevated metal mesh grid and allowed to acclimate for 10 to 20 minutes before testing. Each monofilament was applied 6 times in ascending order to the midplantar region of each hind paw of rat. The monofilament that produced a paw withdrawal, flinch, or lick in 3 of the 6 applications was defined as the 50% paw withdrawal threshold. This test was administered periodically starting on day 0 and ending on day 28. Behavioral experiments were conducted in a quiet behavioral testing room. The behavioral investigator (S.-Y.Y.) was blinded to the treatment of animals during the experiments.

Western Blot

Spinal cord samples were collected from vehicle-treated rats (with normal behavioral phenotype) and oxaliplatin-treated rats (with confirmed phenotype of chemotherapy-induced peripheral neuropathy) at day 7 or day 14 following treatment. Animals were deeply anesthetized with intraperitoneal injection of pentobarbital (90 mg/kg body weight; Lundbeck, Deerfield, IL), and spinal dorsal horn of both sides from L4-6 segment was dissected and quickly frozen in liquid nitrogen and stored at −80°C until further processing. The tissues were then homogenized by sonication in lysis buffer containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2 ethylenediaminetetra-acetic acid, 1 mM ethylene glycol tetra-acetic acid, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, and 1 μg/mL leupeptin, and mixed with protease and phosphatase inhibitor cocktails (Sigma-Aldrich). The protein concentration of the extraction was determined by Lowry protein assay (Bio-Rad, Hercules, CA). Samples (30 μg protein in total) were then heated at 95°C for 5 minutes, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred to a polyvinylidene fluoride membrane using a Transblot SD apparatus (Bio-Rad).

After being washed with twice-buffered saline with Tween-20 (TBST, 10 mM Tris-HCl [pH 7.6], 150 mM NaCl, .05% Tween-20), the blots were blocked with 5% skim milk in TBST at room temperature for 30 minutes and incubated at 4°C overnight with primary antibody, followed by an incubation with horseradish peroxidase-conjugated appropriate secondary antibody at room temperature for 1 hour. The target protein with proper size was detected and visualized by an enhanced chemiluminescence system (Amersham Pharmacia Biotech, Little Chalfont, United Kingdom). Primary antibodies included rabbit anti-Cx43 (1:1000; Invitrogen, Grand Island, NY), rabbit anti-Cx32 rabbit (1:1000; Millipore, Bedford, MA) rabbit anti-Cx36 rabbit