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Participation of pro-inflammatory cytokines in neuropathic pain evoked by chemotherapeutic oxaliplatin via central GABAergic pathway

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Abstract

Background: Neuropathic pain is observed in patients as chemotherapeutic oxaliplatin is used to treat metastatic digestive tumors; however, the mechanisms responsible for hyperalgesia are not well understood. Chronic neuroinflammation is one of the hallmarks of pathophysiology of neuropathic pain. Since the midbrain periaqueductal gray is an important component of the descending inhibitory pathway controlling on central pain transmission, we examined the role for pro-inflammatory cytokines system of the periaqueductal gray in regulating mechanical hyperalgesia and cold hypersensitivity evoked by oxaliplatin.

Methods: Neuropathic pain was induced by intraperitoneal injection of oxaliplatin in rats. ELISA and western blot analysis were used to examine pro-inflammatory cytokine levels and their receptors expression.

Results: IL-1β, IL-6, and TNF-α were elevated within the periaqueductal gray of oxaliplatin rats. Protein expression of IL-1β, IL-6, and TNF-α receptors (namely, IL-1R, IL-6R, and TNFR subtype TNFR1) in the plasma membrane periaqueductal gray of oxaliplatin rats was upregulated, whereas the total expression of pro-inflammatory cytokine receptors was not altered. In oxaliplatin rats, impaired inhibitory gamma-aminobutyric acid within the periaqueductal gray was accompanied with decreases in withdrawal thresholds to mechanical stimulus and % time spent on the cold plate. Our data further showed that the concentrations of gamma-aminobutyric acid were largely restored by blocking those pro-inflammatory cytokine receptors in periaqueductal gray of oxaliplatin rats; and mechanical hyperalgesia and cold hypersensitivity evoked by oxaliplatin were attenuated. Stimulation of gamma-aminobutyric acid receptors in the periaqueductal gray also blunted neuropathic pain in oxaliplatin rats.

Conclusions: Our data suggest that the upregulation of pro-inflammatory cytokines and membrane pro-inflammatory cytokine receptor in the periaqueductal gray of oxaliplatin rats is likely to impair the descending inhibitory pathways in regulating pain transmission and thereby contributes to the development of neuropathic pain after application of chemotherapeutic oxaliplatin.

Keywords

Oxaliplatin, mechanical pain, cold hypersensitivity, cytokines, central gamma-aminobutyric acid

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Introduction

Oxaliplatin (OXL) is an organoplatinum compound, and as a third-generation chemotherapeutic agent, it is commonly used to treat the cancer.[1] Especially, it has a significant activity against advanced and/or metastatic digestive tumors, but one of the main limiting complications of OXL is painful neuropathy.[2] The signs of neuropathy start with paresthesia, followed by

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hyperesthesia.[3] Also, a heightened cold sensitivity is observed in cancer patients with OXL treatment.[2] Overall, treatment options for these abnormal sensations have been restricted, partly due to a poor understanding of the underlying mechanisms responsible for neuropathic pain induced by chemotherapeutic OXL.

As a component of the descending pain modulatory network, the midbrain periaqueductal gray (PAG) has an inhibitory or excitatory control on pain transmission via the rostral ventromedial medulla, projecting to the spinal dorsal horn.[4–6] Accordingly, in this study, we examined the underlying mechanisms by which the changes in neural substrate activity in the PAG are engaged in OXL-induced pain.

Chronic neuroinflammation is one of the hallmarks in regulating neuropathic pain.[7] Studies in neuropathic pain of human patients and experimental animal models show that the activation of glial cells and elevation of pro-inflammatory cytokines (PICs; i.e., IL-1β, IL-6, and TNF-α) levels are common features of neuropathic pain.[8,9] Chronic release of PICs by stimulated astrocytes and microglia leads to the exacerbation of neuronal cells in the pain regulation-related brain regions.[8,9] Infiltration and accumulated immune cells from the periphery are identified in and around the affected brain regions of animal models with chemotherapeutic OXL.[10] Moreover, inflammatory processes have been suggested as promising interventional targets for cancer patients.[9] A better understanding of the role of inflammation in patients treated with OXL will provide new insights into the pathological processes and help to establish effective therapeutic strategies.

Gamma-aminobutyric acid (GABA) is a main inhibitory neurotransmitter in the central nerve system in control of neuronal excitability. After GABA release from presynaptic terminals, GABA transporters play a role in regulating a rapid removal of extracellular GABA,[11,12] which thereby leads to ending of inhibitory synaptic transmission. Thus, this mechanism is responsible for GABA spillover to neighboring synapses[11,13] and GABA homeostasis.[11,14] In contrast, under certain pathological and physiological conditions, the abnormal levels of GABA are observed.[15,16] A recent study suggests that PIC pathways are upregulated in the brain of rats with excitatory neuronal activities, and this alters the expression of GABA via IL-1β and TNF-α receptors.[17]

Therefore, in this study, we determined the levels of IL-1β, IL-6, and TNF-α and their receptors expression (IL-1R, IL-6R, and TNFR1) in the PAG tissues of OXL rats and control rats. Also, we examined if PIC pathways are involved in pain responses evoked by OXL via the descending pain modulatory mechanisms. We hypothesized that the levels of PICs and protein expression of PIC receptors are upregulated in the PAG of OXL rats and blocking PIC signals in the PAG attenuates mechanical hyperalgesia and cold hypersensitivity after the administration of OXL via GABAergic inhibitory pathways.

Materials and methods

Animals

All animal protocols were in accordance with the guidelines of the International Association for the Study of Pain and approved by the Institutional Animal Care and Use Committee of Jilin University. Adult male Sprague-Dawley rats (200–250 g) were housed in individual cages with free access to food and water and were kept in a temperature-controlled room (25°C) on a 12/12 h light/dark cycle.

A model of neuropathic pain

OXL (Tocris Biosci) was dissolved in a 5% glucose solution at a final concentration of 2 mg/ml. Acute neurotoxicity was induced in rats by a single intraperitoneal (i.p.) injection of OXL (6 mg/kg), as described previously.[18,19] Control rats received the same volume of i.p. injection of glucose vehicle. Mechanical and cold hypersensitivity were fully developed by OXL three days after injection (Figure 1) and experiments were performed.

PAG cannulation and drug infusion

Three days were allowed before the experiments. Rats were implanted with a stainless steel guide cannula (0.8 mm once daily) with sodium pentobarbital (60 mg/kg, i.p.), and then the guide cannula was secured to the skull. Stereotaxic coordinates for the dorsolateral PAG (dl-PAG) were 7.6 mm posterior to the bregma, 0.65 mm lateral to the midline, and 4.2 mm ventral to the brain surface.

Following this, cannula was connected to an osmotic minipump (Alzet pump brain infusion kit, DURECT Inc., Cupertino, CA) with polycarbonate tubing. The pumps were placed subcutaneously between the scapulae and loaded with vehicle (artificial cerebrospinal fluid) as control or each of PIC receptor antagonists, namely IL-1Ra (IL-1β receptor antagonist) and SC144 (gp130 antagonist to block IL-6R) and etanercept (ETAN; TNF-α receptor antagonist), respectively (Tocris Co., Ellisville, MO). In a subgroup, muscimol, agonist of GABAa receptors was loaded. The PIC receptor antagonists in 10 μM of concentration and muscimol in 100 μM of concentration were delivered at 0.25 μl per hour (Alzet Model 1003D/3 day delivery; DURECT Inc., Cupertino, CA). This intervention allowed animals to receive continuous PAG infusion via the osmotic minipumps before the experiments and brain tissues were

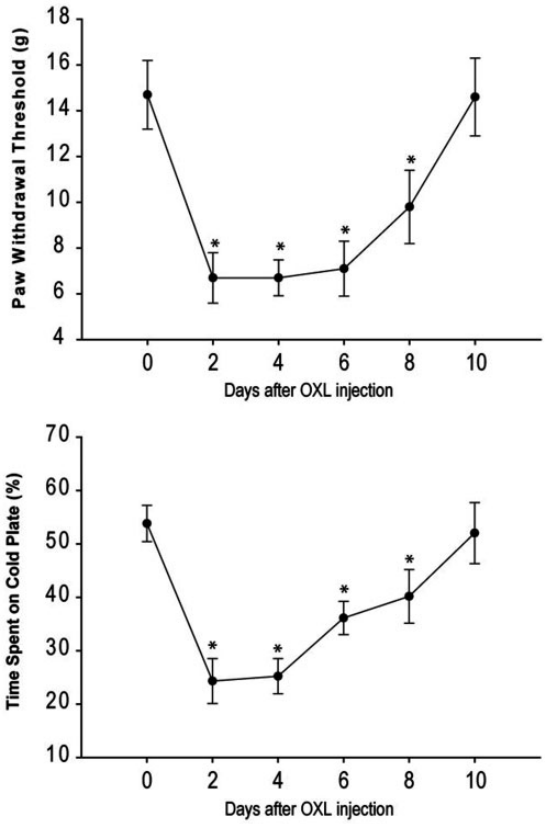

Figure 1. Time course of OXL-induced neuropathic pain. Mechanical and cold hypersensitivity appeared 2 days and lasted approximately 10 days after a signal injection of OXL (6 mg/kg). The peak response induced by OXL is approximately two to four days after its injection. $^*P < 0.05$ versus prior injection of OXL. The number of rats = 12.
OXL: oxaliplatin.

taken out. Note that all drugs were dissolved in artificial cerebrospinal fluid as a final concentration.

Behavioral test

To quantify the mechanical sensitivity of the hindpaw, rats were placed in individual plastic boxes and allowed to acclimate for >30 min. Mechanical paw withdrawal threshold (PWT) of rat hindpaw in response to the stimulation of von Frey filaments was determined. A series of calibrated von Frey filaments (ranging from 0.5 to 18.0 g) were applied perpendicularly to the plantar surface of the hindpaw with a sufficient force to bend the filaments or until paw withdrew. In the presence of a response, the filament of next lower force was applied. In the absence of a response, the filament of next greater force was applied. To avoid injury during tests, the cutoff strength of the von Frey filament was 18 g. The tactile stimulus producing a 50% likelihood of withdrawal was determined using the "up-down" method.[20] Each trial was repeated two times at approximately 2-min intervals. The mean value was used as the force produced a withdrawal response.

To examine cold sensitivity, Thermal Place Preference System (Coulburn Instruments) was used to perform the thermal place preference test in order to assess a cold avoidance behavior. Two connecting metal plates were surrounded by a plastic enclosure. The first plate was kept at neutral temperature (25°C) and the second plate was kept at cold temperature (12°C). The test was performed in darkness, and each session lasted 3 min. During the session, the rats were left free to explore both plates. The time spent on the cold plate during the entire session was recorded using an infrared camera connected to a computer to determine cold avoidance behavior. To better control behavior test, the rats were repeatedly placed on the apparatus with both plates held at room temperature (25°C) during 3 min two days before the beginning of the experiment. Note that the rats spent an equal amount of time on each plate under these conditions, suggesting that the animals showed no place preference. Also, to avoid learning or any place preference unrelated to cold, the temperature of the plates were inverted between two consecutive sessions. Two trials were performed for each of the drugs and data were averaged. It is noted that all behavioral tests were performed in a blind fashion.

At the end of the experiments, 2% Evans blue in 0.25 μl was infused through the cannula. Then, the animals were anesthetized by sodium pentobarbital and intracardiacally perfused with physiological saline followed by 4% of paraformaldehyde solution. The midbrain was sectioned, and the location of injection sites was verified by histological examination of blue dye according to the atlas of Swanson.[21] The rats with microinjection site was localized within the dl-PAG were included for data analysis.

ELISA measurements

The rats were first euthanized by overdose sodium pentobarbital (120 mg/kg, i.p.), and then the dorsolateral regions of PAG were dissected under an anatomical microscope. Total protein of the PAG tissue was then extracted by homogenizing sample in ice-cold radioimmunoprecipitation assay buffer with protease inhibitor cocktail kit. The lysates were centrifuged and the supernatants were collected for measurements of protein concentrations using a bicinchoninic acid assay reagent kit. The levels of IL-1β, IL-6, and TNF-α were examined using an ELISA assay kit (Promega Corp) corresponding to the provided description and modification. Briefly, polystyrene 96-well microtitel immunoplates were coated with affinity-purified polyclonal rabbit anti-IL-1β, anti-IL-6, and anti-TNF-α antibodies, respectively. Parallel wells were coated with purified rabbit IgG for the

evaluation of nonspecificity. After overnight incubation, the diluted samples and the PICs standard solutions were distributed in each plate. The plates were washed and incubated with anti-IL-1β, anti-IL-6, and anti-TNF-α galactosidase, respectively. Then, the plates were washed and incubated with substrate solution. After incubation, the optical density was determined using an ELISA reader. In the similar way, the levels of GABA were determined (LDN Diagnostics, Inc., Colorado Springs, CO) according to the provided description and modification.

Western blot analysis

Similar to the ELISA, dl-PAG tissues were removed. In order to determine the expression of PIC receptors on cell surface, PAG tissues were incubated with Sulfo-NHS-LC-Biotin (1 mg/ml, Pierce) for 30 min on ice as described previously.[22] Because biotin is impermeable to the cell membrane, only proteins on the cell surface were biotinylated. The unbound biotin in the solution was removed by washing PAG tissues five times. PAG tissues were then homogenized and centrifuged at 13,500*g* (4°C) for 12 min. A sample (200 μg protein) was incubated with streptavidin beads (20 μl) for 3 h at 4°C. The beads were washed three times with radioimmunoprecipitation assay buffer and precipitated by centrifugation and collected. Sample buffer (50 μl) was added to the collected beads and boiled for 3 min. Beads were pelleted again by centrifugation and the supernatant was collected. The supernatant was diluted to the same volume as the starting material (i.e., 200 μg total protein). Total and membrane samples in equal volume were applied to sodium dodecyl sulfate polyacrylamide gel electrophoresis. Membranes were incubated with the rabbit anti-IL-1R, anti-IL-6R, and anti-TNFR1 primary antibodies (1:500, obtained from Neuromics and Abcam Co). After being fully washed, the membrane was incubated with horseradish peroxidase-linked anti-rabbit secondary antibody (1:250) and visualized for immunoreactivity. The membrane was also processed to detect β-actin for equal loading. The bands recognized by the primary antibody were visualized by exposure of the membrane onto an X-ray film. The film was then scanned and the optical densities of protein bands were analyzed using the Scion image software. Then, the values for densities of immunoreactive bands/β-actin band from the same lane were determined. Each of the values was then normalized to a control sample.

Statistical analysis

All data were analyzed using a one-way analysis of variance. As appropriate, Tukey's post hoc analyses were utilized to determine differences between groups. Values were presented as means ± standard error. For all analyses, differences were considered significant at $P < 0.05$. All statistical analyses were performed by using SPSS for Windows version 13.0 (SPSS Inc., Chicago, IL).

Results

Time course of OXL-induced neuropathic pain

Previous studies demonstrated that mechanical and thermal pain appeared 2 days and lasted approximately 10 days after a signal injection of OXL (i.e., 6 mg/kg).[18,19] The peak response induced by OXL is approximately two to four days after its injection. In this study, we observed the same results for the time course in agreement with those previous reports as shown in Figure 1. Thus, three days (after injection of OXL) were selected in this report to examine mechanical and cold sensitivity and the effects of PIC and GABA signals. In control rats, glucose failed to alter mechanical and cold sensitivity.

Levels of PICs and expression of PIC receptors

We examined the levels of PICs as well as total protein and membrane expression of PIC receptors in the dl-PAG of control rats (n = 18) and OXL rats (n = 20). Figure 2(a) showed that IL-1β, IL-6, and TNF-α were elevated in OXL rats as compared with control animals. Figure 2(b) and (c) demonstrated that the total PIC receptors expression in the PAG was not significantly altered in OXL rats, but membrane PIC receptors expression was significantly increased in OXL rats as compared with control animals. Figure 2(d) further showed that the ratio of membrane and total PIC receptor densities was greater in the PAG of OXL rats than that of control rats. The ratio of membrane protein and total protein for IL-1R, IL-6R, and TNFR1 was 1.69 ± 0.20, 1.63 ± 0.21, and 1.60 ± 0.0.2, respectively, in the PAG of PD rats ($P < 0.05$ vs. their respective controls).

Pain responses to mechanical and cold stimuli

PWT and % time spent on the cold plate appeared to be less in OXL animals (n = 15; $P < 0.05$ vs. control rats) as compared with control rats (n = 10). We further examined the effects of blocking PIC receptors (respective IL-1R, IL-6R, and TNFR1) in the dl-PAG on PWT and % time spent on the cold plate in OXL rats (n = 8–12 in each group). Figure 3 demonstrated that PWT (top panels) and % time spent on the cold plate (bottom panels) were significantly increased during a 40-min period of the test with a 20-min interval after blocking each of PIC receptors ($P < 0.05$ vs. OXL rats). Note that there were no differences in PWT and % time spent on

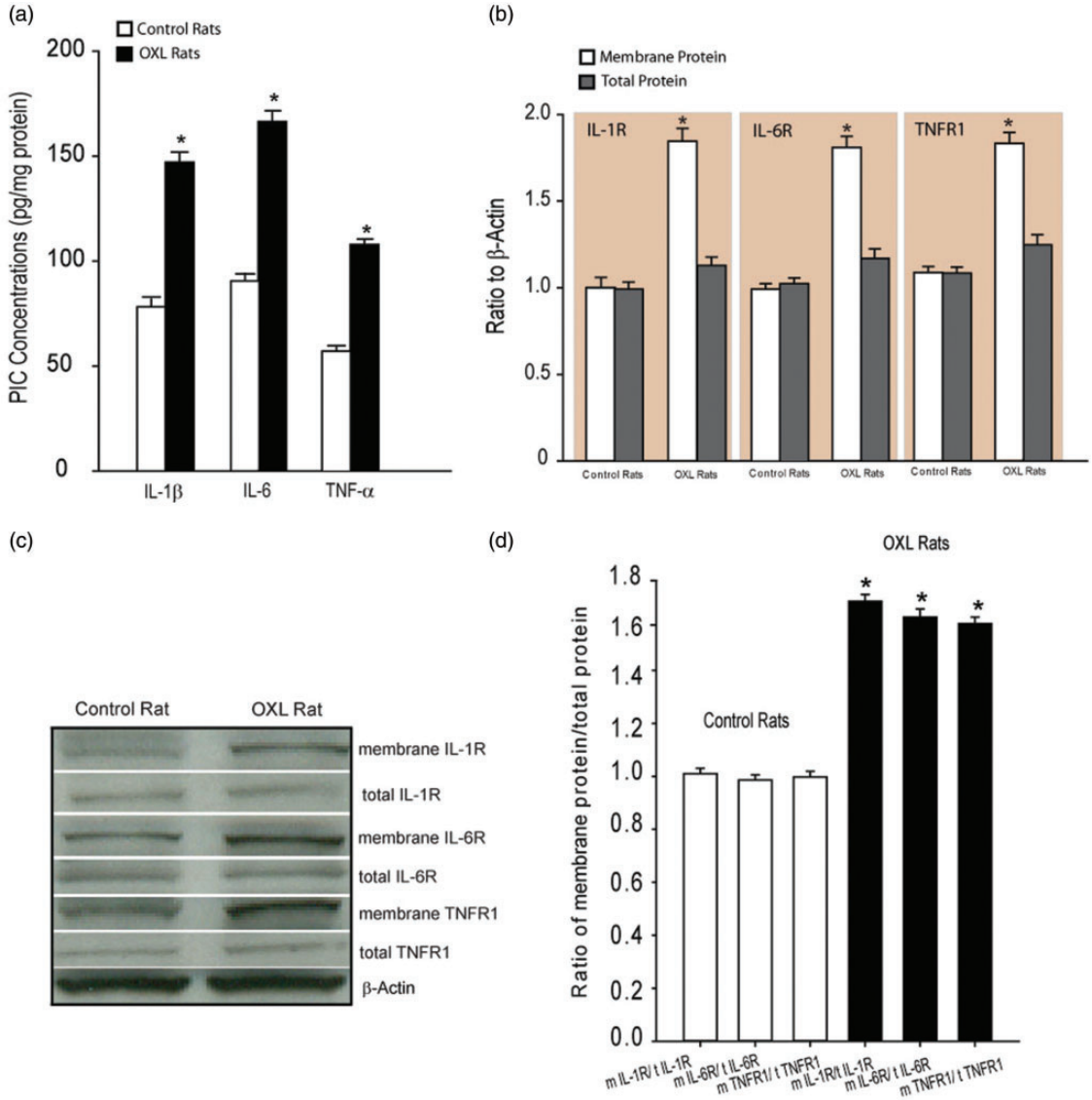

Figure 2. PIC signal in the dl-PAG. (a) The levels of PICs in the dl-PAG of control rats and OXL rats. (b and c) Averaged data and typical bands showing the protein expression of PIC receptors (IL-1R, IL-6R, and TNFR1). Membrane PIC receptors are increased in OXL rats, whereas total protein expression is not significantly altered. (d) The ratio of membrane PIC receptors protein/total PIC receptors protein. *$P < 0.05$ versus control rats. The number of control rats = 18 and the number of OXL rats = 20.
OXL: oxaliplatin; PIC: pro-inflammatory cytokine.

the cold plate between controls and OXL rats with PIC receptors blocking ($P > 0.05$, OXL rats with PIC inhibitors vs. control rats).

Engagement of GABA

Figure 4(a) demonstrated that the levels of GABA were significantly decreased in the dl-PAG of OXL rats compared with control animals ($P < 0.05$, OXL rats/n = 15 vs. control rats/n = 12). With infusion of respective PIC receptor antagonists lessened GABA was restored (n = 8 in each group, $P < 0.05$ vs. OXL rats), but no significant differences were observed in GABA levels between control animals and OXL animals with PIC receptors blocking ($P > 0.05$ vs. control rats).

We further examined the effects of the stimulation of GABAa by the infusion of muscimol in the dl-PAG on PWT and % time spent on the cold plate in OXL rats. Figure 4(b) and (c) showed that PWT and % time spent on the cold plate were significantly increased during a 40-min period of the test with a 20-min interval after the stimulation of GABAa in OXL rats ($P < 0.05$ vs. OXL rats, n = 10 in OXL rats and n = 12 in OXL rats with muscimol). No significant differences in PWT and % time spent on the cold plate were observed between controls and OXL rats with muscimol ($P > 0.05$ vs. control

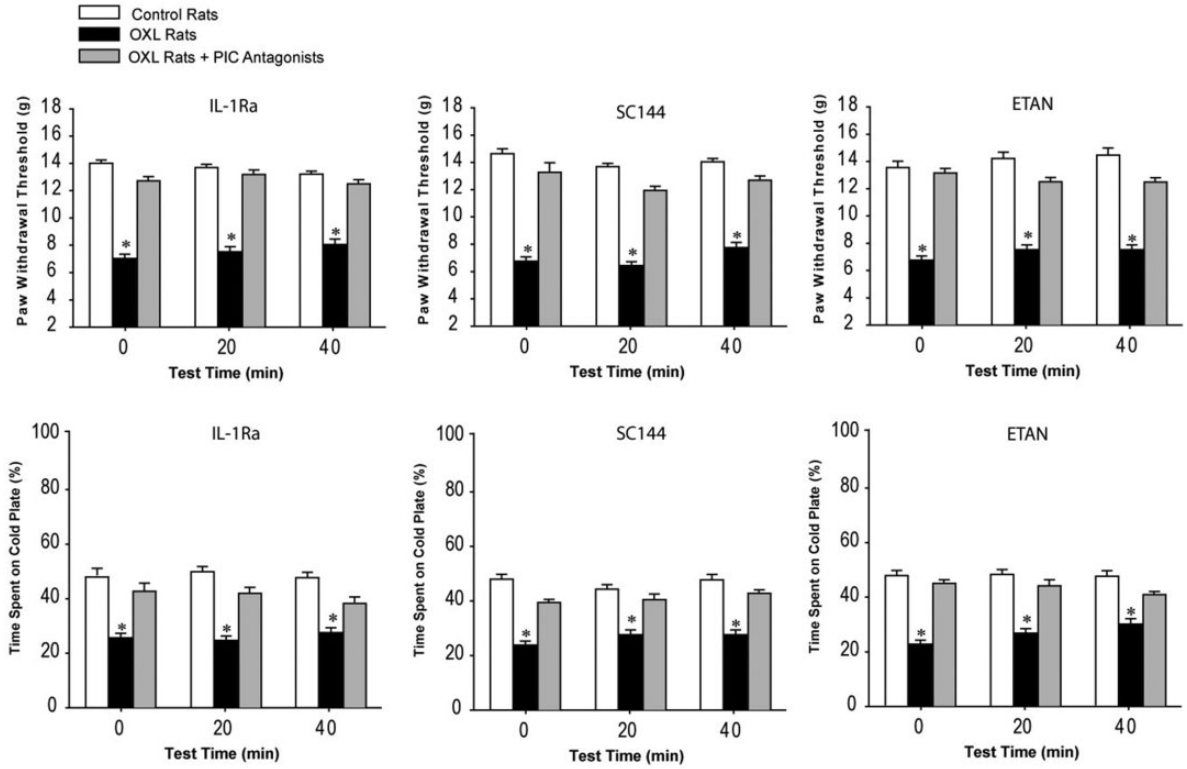

Figure 3. Effects of blocking PIC receptors on mechanical and cold hyperalgesia. Effects of blocking PIC receptors in the dl-PAG on pain responses to mechanical and cold stimulation. Mechanical and cold hyperalgesia appeared to be less in OXL rats (n = 15) as compared with control animals (n = 10). Infusion of respective PIC receptor inhibitors into the PAG attenuated hypersensitive responses in OXL rats (n = 8 for IL-1Ra, n = 10 for SC144, and n = 12 for ETAN). *$P < 0.05$ versus control rats and OXL rats that received infusion of inhibitors over a 40-min testing time.
OXL: oxaliplatin; PIC: pro-inflammatory cytokine.

rats, n = 8). This result suggests the engagement of GABA in hypersensitive mechanical and cold responses in OXL rats.

Discussion

Overall, the main findings of this study are that (1) IL-1β, IL-6, and TNF-α and their receptors in membrane expression are upregulated in the dl-PAG of OXL rats and (2) blocking those individual receptors in this brain region attenuates hypersensitive responses to mechanical and cold stimuli in OXL rats likely by improving impaired GABAergic descending inhibitory system.

One of the most common and distressing symptoms suffered by patients with progression of cancer is pain.[23] Cancer pain mainly arises from a tumor compressing or infiltrating tissue; from nerve and other changes caused by a hormone imbalance or immune response; and/or from treatments and diagnostic procedures.[3,23] It should be noted that chemotherapy (i.e., OXL) and radiotherapy may produce painful conditions that persist long after the treatment has ended.[23–25] As a result, how to effectively manage cancer pain related to these therapies becomes an important issue for the treatment and the management of cancer patients in clinics.

Evidence has suggested that antinociception is mediated partly by descending pathways arising from the midbrain PAG.[26,27] Early studies showed that electrical stimulation or opioids microinjected into the PAG produced profound long-lasting antinociception.[26,27] In particular, activated neuronal cells are identified in the brain of macaques with the administration of OXL,[28] suggesting neural substrates are likely present within the PAG in engagement of the abnormalities in pain response observed after OXL. Furthermore, previous studies showed that PIC mediators appear in the PAG, and the activation of PICs in the PAG plays a role in modulating pain response or is involved in morphine withdrawal response.[29,30] Nonetheless, to the best of our knowledge, data of this study have shown for the first time that PIC signal pathways in the PAG plays a role in regulating abnormal mechanical and cold responses in a rat model of OXL-induced neuropathy.

It is well-known that IL-1β is involved in the immune response and signal transduction both in the periphery and the central nervous system.[31] IL-1β produced in the

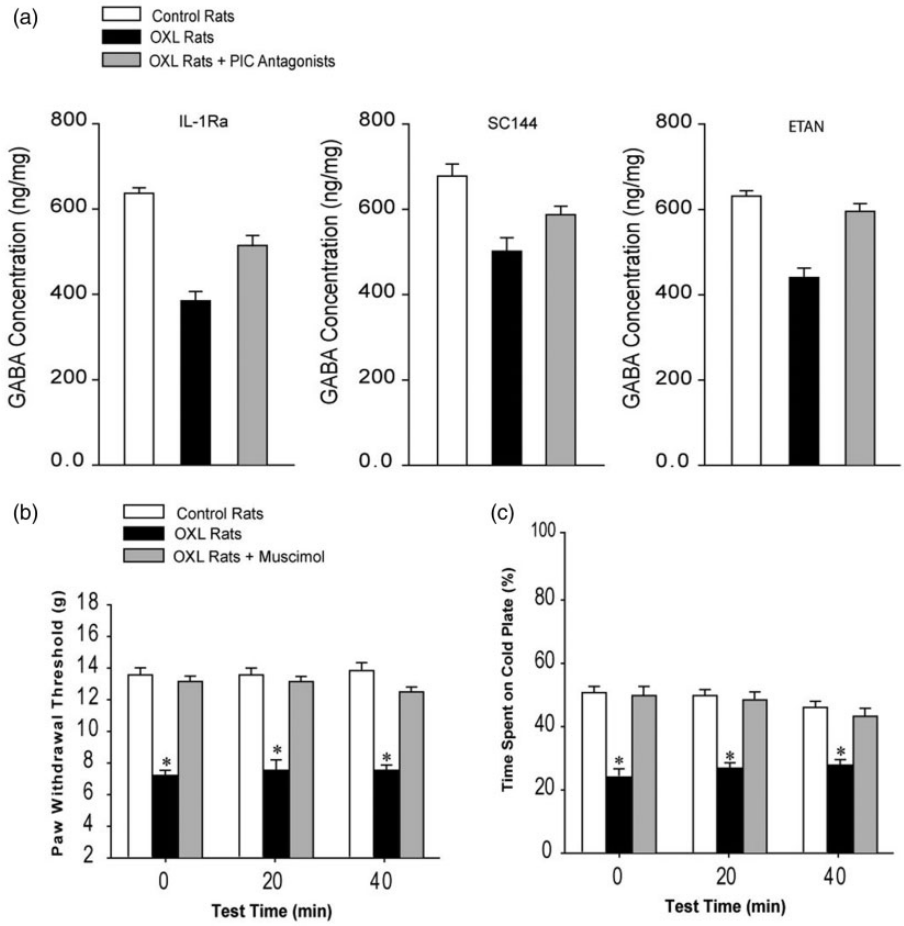

Figure 4. Involvement of GABA in the effects of PICs. (a) The levels of GABA in the dl-PAG in control rats and OXL rats. The GABA was significantly diminished in OXL rats (n = 15) as compared with control animals (n = 12). Injection of respective PIC receptor inhibitors largely restored impaired GABA. *$P < 0.05$ versus control rats and rats with infusion of PIC receptor inhibitors (n = 8 in each group). (b and c) Effects of stimulation of GABAa receptors in the dl-PAG on pain responses to mechanical and cold stimulation. Mechanical and cold hyperalgesia appeared to be less in OXL rats (n = 10) as compared with control animals (n = 8). Infusion of GABAa receptor agonist, muscimol into the PAG attenuated hypersensitive responses in OXL rats (n = 12). *$P < 0.05$ versus control rats and OXL rats with infusion of muscimol over a 40-min testing time.
OXL: oxaliplatin; PIC: pro-inflammatory cytokine.

nervous system regulates the function of neuron and glia cells.[32] Prior studies specifically demonstrated that IL-1β contributes to inflammatory and neuropathic pain.[33] Increased level of IL-1β has been observed in the cerebrospinal fluid of chronic pain patients[34] and in the brainstem, contralateral thalamus/striatum, and prefrontal cortex of rats with spared nerve injury.[35] A prior study showed that the inhibition of melanocortin 4 receptor in the PAG blunts mechanical allodynia and thermal hyperalgesia but also delays the development of pain facilitation induced by peripheral nerve injury.[36] This further decreases the expression of levels of IL-1β, IL-6, and TNF-α.[36] Treatments with anti-IL-1β neutralizing antibodies or with IL-1Ra have also been reported to attenuate or block the hyperalgesia induced by various nociceptive injuries.[33,37] Consistent with these prior findings, in this study, we found that membrane expression of IL-1R was increased in the dl-PAG of OXL rats and blocking IL-1R in this brain region attenuated hypersensitive responses to mechanical and cold stimuli in OXL rats.

IL-6 complexes with membrane-bound or soluble IL-6R to stimulate cells expressing the signal transducer glycoprotein (gp130).[38,39] Most cells are lacking of membrane-bound IL-6R and are thus unresponsive to IL-6. Nevertheless, they still react to IL-6 complexed with a soluble form of the IL-6R (sIL-6R) to activate gp130, a pathway called "trans-signaling."[38] Thus, in this study, we used SC144, a gp130 inhibitor, to block IL-6-mediated signal transduction in order to examine the engagement of the IL-6R in GABAergic signals and pain response thresholds to mechanical and cold stimuli

in OXL rats. Our data showed that IL-6R was upregulated by OXL and SC144 injected into the PAG improved impairment of GABA and attenuated mechanical and cold hypersensitivity induced by OXL.

The effects of TNF-α are via stimulation of two TNF-α receptor subtypes, TNFR1 and TNFR2.[40] TNFR1 is present entirely on neuronal cells and plays a functional role, whereas TNFR2 is located predominantly on macrophages and/or monocytes in response to inflammation.[41] Thus, in this study application of ETAN lessens GABA in the PAG of OXL rats and attenuates pain response, it is likely via TNFR1. In addition, we observed distinct expression of TNFR1 receptors in the PAG of OXL rats.

In this study, we demonstrated that cell membrane PIC receptors are upregulated in the dl-PAG of OXL rats. However, the total protein expression of PIC receptors was not considerably altered in the PAG of OXL rats, indicating that PIC receptors trafficking to the cell membrane of PAG is particularly amplified in OXL rats.[22] The underlying mechanism for the increase in trafficking of PIC receptors following OXL injection needs to be determined. The elevated PICs were also observed in OXL rats in this study. Accordingly, we assume that PICs are likely released from the glial cells and this signal is likely to lead to the upregulation of membrane PIC receptors. Nevertheless, it is speculated that the increased activities in the PIC pathways are likely to result in neuronal damage within the PAG since PICs are engaged in the process of apoptosis, which has been observed in brains.[42]

In the central nervous system (CNS), glutamate and GABA play a dominant role in regulating neuronal functions; one is excitatory and another is inhibitory.[43] Adrenalin and 5-HT, and so on are also neurotransmitters mediating the descending pain response likely via altering excitatory glutamate and/or inhibitory GABA mechanisms. Interestingly, the results of this study demonstrated that the levels of GABA were significantly decreased in the dl-PAG of OXL rats, indicating that GABA is impaired as a part of major descending inhibitory pathways. The increased PICs are likely to damage neurons of the dl-PAG thereby leading to a reduction in GABA. There are some possibilities that PICs and/or the activation of PIC receptors can alter GABAergic pathways.[44] Prior studies showed that the stimulation of this region of PAG led to antinociceptive effects.[4,5] This supports our hypothesis that the activation of PIC receptors within the PAG plays a de-inhibitory role in regulating the descending pain pathways. When PIC receptors are blocked in the dl-PAG, the abnormal descending pain pathways are largely restored because we have observed that chronic infusion of PIC antagonists lessened amplified pain responses in OXL animals, accompanied with increasing GABA levels in the PAG. Consistent with this result, our results also found that mechanical and cold hyperalgesia in OXL rats were attenuated following the stimulation of GABAa receptors in the dl-PAG by infusion of muscimol. Overall, this suggests that the activation of PIC signals influences GABAergic transmission within this region of PAG and thereby amplifies pain response.

Study limitations

Numerous studies have shown that PICs and their receptors are constitutively expressed by neuronal and glial cells (i.e., microglia and astrocytes) in the CNS and influence neuronal functions.[44] The focus of this study was to examine the role played by PICs (such as IL-1β, IL-6, and TNF-α) of the dl-PAG in regulating neuropathic pain responses induced by OXL. Thus, we examine the levels of PICs and their receptor expression in the dl-PAG of control rats and in OXL rats. It is well reasoned that PICs and their receptors appeared in neuronal and glial cells. However, a limitation of this study was that we cannot differentiate PICs and their receptors in neuronal cells and/or glial cells by using ELISA and western blot analysis. In addition, as a major inhibitory neurotransmitter, GABA is made in neuronal cells and released from the cell bodies to terminals/other local neurons to play a functional role.[43] Thus, in this report, we did not determine the cell types containing GABA since GABA appears most likely within the neuronal cells.

In conclusion, we have shown that PIC signal pathways are activated in the dl-PAG of OXL rats and thereby deinhibit GABAergic-mediated descending regulation in pain transmission. These abnormalities are likely to contribute to the development of mechanical and cold hypersensitivity in OXL animals. Blocking PIC receptors inhibits neuropathic pain induced by the administration of OXL. Results of this study provided a base for the mechanisms responsible for OXL-induced pain. This further offers promising clues to target CNS for the development of new therapeutic strategies for managing intractable pain response in cancer patients with chemotherapeutic OXL.

Author Contributions

Dongsheng Xu and Hui Zhao contributed to experimental performance, data analysis, and drafting the manuscript, and they had equal contributions to this work as co-first authors. Han Gao and Huiling Zhao also participated in experimental performance and data analysis. Dandan Liu and Jing Li designed and oversaw the experiments and reviewed the manuscript, and they are corresponding authors.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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