

# Analgesic effect of AG490, a Janus kinase inhibitor, on oxaliplatin-induced acute neuropathic pain

Shuang-Feng Li<sup>1,2</sup>, Bi-Shan Ouyang<sup>2</sup>, Xin Zhao<sup>3</sup>, Ya-Ping Wang<sup>1,\*</sup>

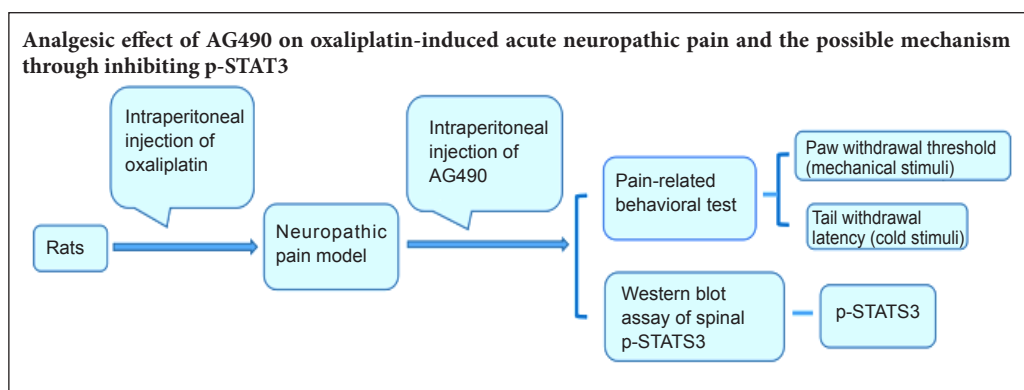
<sup>1</sup> Department of Anesthesiology, The Second Xiangya Hospital, Xiangya School of Medicine, Central South University, Changsha, Hunan Province, China

<sup>2</sup> Department of Anesthesiology, Hainan General Hospital, Haikou, Hainan Province, China

<sup>3</sup> Department of Neurology, Xiangya Hospital, Xiangya School of Medicine, Central South University, Changsha, Hunan Province, China

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## Graphical Abstract



**\*Correspondence to:**

Ya-Ping Wang, Ph.D.,

Wangyaping6568@csu.edu.cn.

**orcid:**

0000-0001-6010-095X

(Ya-Ping Wang)

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## Abstract

Neuropathic pain often occurs during chemotherapy with oxaliplatin. AG490 has been shown to exert an antagonistic effect on inflammatory pain, but its effect on oxaliplatin-induced neuropathic pain remains poorly understood. This study sought to observe the analgesic effect of AG490 on acute neuropathic pain induced by a single oxaliplatin treatment and to address the possible mechanism. In this study, we established a model of oxaliplatin-induced acute neuropathic pain by intraperitoneal injection of 6 mg/kg oxaliplatin. On day 2 after injection, models were intraperitoneally injected with 1, 5, or 10 mg/kg AG490. Paw withdrawal threshold to mechanical stimuli and tail withdrawal latency to cold stimuli were determined. Western blot assay was performed to detect the expression of spinal phosphorylated signal transducer and activator of transcription 3 (p-STAT3). Immunohistochemistry was used to determine the immunoreactivity of p-STAT3 and interleukin-6. Results demonstrated that paw withdrawal threshold and tail withdrawal latency were significantly increased by the treatment of AG490 in rats. There was no significant difference in the effect among the different doses of AG490. AG490 10 mg/kg decreased the expression of p-STAT3, the immunoreactivity of p-STAT3 and interleukin-6 in spinal cord of acute neuropathic pain rats. These findings confirm that AG490 can attenuate oxaliplatin-induced acute neuropathic pain and is associated with the inhibition in the JAK/STAT3 signaling pathway.

**Key Words:** nerve regeneration; AG490; analgesia; oxaliplatin; cancer chemotherapy; neuropathic pain; phosphorylated STAT3; JAK/STAT signaling pathway; interleukin-6; neural regeneration

## Introduction

Oxaliplatin, a chemotherapeutic agent, often causes adverse side effects, such as diarrhea, emesis, muscle pain, joint pain, and neuropathic pain (Argyriou et al., 2013). Neuropathic pain is one of the most severe adverse side effects during cancer chemotherapy, and controlling the symptoms of neuropathic pain is a major clinical problem (Argyriou et al., 2013). There are no clear alternative interventions for the effective treatment of neuropathic pain in patients that are refractory to available opioid and non-opioid analgesics (Inoue et al., 2011; Nagashima et al., 2014), or who develop severe side effects that result in withdrawal from chemotherapy. These concerns have prompted the identification of novel targets and experimental strategies to test potent interventions in well-validated animal models of neuropathic pain.

The signal transducer and activator of transcription 3 (STAT3) protein is a prominent cascade for cellular signal transduction. Several studies have shown that STAT3 is activated after nerve injury in animal models of neuropathic pain, and that it is present in multiple cell types (Yamauchi et al., 2006; Dominguez et al., 2008, 2010; Wang et al., 2014; Popiolek-Barczyk et al., 2015). Inhibition of microglial JAK/STAT3 signaling attenuated mechanical allodynia in neuropathy induced by ligation of spinal nerves in rats (Dominguez et al., 2008). AG490 is a synthetic derivative of benzylidenemalonitrile and is a specific and potent inhibitor of JAK/STAT signaling (Levitzi, 1990). The oxaliplatin-induced acute neuropathic pain model in rats is widely used to study acute neuropathic pain or the effect of analgesic drugs (Ling et al., 2007; Lim et al., 2013). A study has shown that

AG490 possesses an antiallodynic effect on inflammatory pain (Cheppudira et al., 2015). Therefore, we studied the analgesic effect of AG490 on acute neuropathic pain induced by a single oxaliplatin treatment in rats and addressed the possible mechanism.

## Materials and Methods

### Animals

A total of 56 12-week-old specific-pathogen-free male Sprague-Dawley rats were purchased from Tianqin Biotechnology Company in China (license No. SCXK (Xiang) 2014-0010). Experimental animals were raised in clean facilities with free access to food and water. Rats were housed in a room maintained at  $22 \pm 1^\circ\text{C}$  with a 12-hour light-dark cycle (lights on 8:00 a.m. to 8:00 p.m.). The animal experiment was conducted in accordance with animal protocols approved by the Institutional Animal Care and Use Committee of Xiangya Research Laboratory, China (approval No. IACUC-2016-0157). Every effort was made to minimize the numbers and any suffering of animals used in the study. Rats were habituated to handling by investigators and to all testing procedures for a week before starting the experiments. The researchers performing the behavioral studies were blinded to the treatment administered.

### Model establishment

An oxaliplatin-induced acute neuropathic pain model was used in this study. Sixteen rats randomly received either one intraperitoneal injection of oxaliplatin (Hengrui Medicine, Lianyungang, Jiangsu Province, China; 6 mg/kg,  $n = 8$ ) or 5% glucose solution (6 mg/kg,  $n = 8$ ) (Ling et al., 2007; Lim et al., 2013). All behavioral tests were performed 0, 1, 2, 3, 4, and 5 days after the administration of oxaliplatin or 5% glucose. The rats exhibited the most significant increase in mechanical and cold allodynia on day 2. Thus, we selected this time point for subsequent experiments.

### Drug administration

An additional 40 rats were randomly assigned to five groups. The control group received the 5% glucose solution injection. The other four groups received an injection of 6 mg/kg oxaliplatin. AG490 (Sigma, St. Louis, MO, USA) was dissolved in 3.5% dimethyl sulfoxide. Two days after the rats had received the oxaliplatin they were randomly and equally divided into four groups, and received a single injection of either AG490 (1, 5 or 10 mg/kg, intraperitoneally) or vehicle (3.5% dimethyl sulfoxide, intraperitoneally). All rats were euthanized by an overdose of pentobarbital sodium at the end of the experiment.

### Behavioral tests

Behavioral tests representing different sensory components of neuropathic pain were performed 0, 1, 2, 3, 4, and 5 days after oxaliplatin administration, and 4 hours after AG490 injection.

Thermal allodynia was assessed using the tail-immersion test in water maintained at low ( $10^\circ\text{C}$ ) or high ( $42^\circ\text{C}$ ) temperature (Ling et al., 2007). The tails of the rats were immersed in water maintained at the specified temperatures until the tail was withdrawn. The duration of tail immersion was recorded

and a cut-off time of 20 seconds was used. Mechanical allodynia was measured by using the electronic von Frey pain measuring instrument (IITC Life Science, Woodland Hills, CA, USA). A punctate stimulus was delivered to the mid-plantar area of each posterior paw below the mesh floor with a rigid von Frey fiber, and the withdrawal threshold was automatically displayed on the screen when the paw was withdrawn. Stimuli were applied to each posterior paw at 5-second intervals. The cut-off threshold was recorded as 80 g. All measurements were repeated five times and the mean value was obtained.

### Western blot assay

Immediately following the behavioral tests on the indicated days, rats were deeply anesthetized with pentobarbital (100 mg/kg, intraperitoneally). The lumbar enlargements of the spinal cord were removed immediately and placed into liquid nitrogen for rapid freezing. The tissue was put into radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitor cocktails (Sigma-Aldrich) to be homogenized on ice. Following centrifugation at  $4^\circ\text{C}$ , the supernatants were collected. Proteins in the samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrotransferred onto polyvinylidene fluoride membrane (Merck Millipore, Darmstadt, Hesse-Darmstadt, Germany). The membrane was blocked by 5% non-fat milk for 1 hour at room temperature, then incubated overnight at  $4^\circ\text{C}$  with the primary antibodies for phosphorylated STAT3 (Tyr705: rabbit polyclonal antibody IgG; 1:500; Cell Signaling Technology, Boston, MA, USA) and  $\alpha$ -tubulin (rabbit polyclonal antibody IgG; 1:500; Cell Signaling Technology, Boston, MA, USA). The membrane was then incubated with the secondary antibodies (horseradish-peroxidase goat anti-rabbit IgG; 1:3000; Bioworld Technology, St. Louis Park, MN, USA) for 1.5 hours at room temperature, developed in enhanced chemiluminescence solution and exposed onto X-ray film. The density values were computed using Gel-Pro analyzer 4 software (Media Cybernetics, Bethesda, MD, USA) and were standardized with  $\alpha$ -tubulin expression.

### Immunohistochemistry

Rats were deeply anaesthetized by intraperitoneal injection of pentobarbital (100 mg/kg). Cold saline followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.2–7.4,  $4^\circ\text{C}$ ) were perfused through the ascending aorta. Following perfusion, the spinal cord lumbar enlargement was quickly removed, post-fixed in the same fixative for 2 hours, and cytoprotected in 30% sucrose for two nights. Transverse spinal sections ( $5 \mu\text{m}$ ) were cut in a cryostat, mounted on poly-lysine-coated slides and processed for immunohistochemistry/immunofluorescence. All the sections were blocked with 2% goat or donkey serum in 0.1 M Tris-buffered saline/0.3% Triton X-100 for 1 hour at room temperature. First, the tissue sections were mounted in 4',6-diamidino-2-phenylindole solution (1.0 mg/mL; Solarbio, Beijing, China) for dyeing. Second, the tissue sections were incubated over two nights at  $4^\circ\text{C}$  with either the primary antibody for p-STAT3 (Tyr705; rabbit polyclonal antibody IgG; 1:500; Cell Signaling Technology, Boston, MA, USA) or interleukin-6 (IL-6) (mouse monoclonal IgG; 1:2000; Abcam Plc, Cambridge, MA, USA). Following incubation, the tissue

sections were washed and incubated for 3 hours at room temperature in a secondary antibody solution (Alexa Fluor 594, goat anti-mouse IgG; 1:1000; Molecular Probes, Eugene, OR, USA). The stained sections were observed using a fluorescence microscope (Olympus, Tokyo, Japan), and the images were captured by a CCD spot Camera.

### Statistical analysis

Data are presented as the mean  $\pm$  standard error of mean. Statistical evaluation was performed with SPSS 22.0 software (IBM SPSS, Chicago, IL, USA). Data from the experiments were validated using one-way analysis of variance, followed by Tukey's multiple comparison. A value of  $P < 0.05$  was considered statistically significant.

## Results

### Influence of oxaliplatin on cold, heat and mechanical allodynia and expression of spinal p-STAT3 in rats

The effects of a single administration of oxaliplatin (6 mg/kg, intraperitoneally) was investigated on behavioral tests and on the JAK/STAT3 signaling in rats. Before oxaliplatin injection, there were no significant differences in mean thresholds between the oxaliplatin-treated group (6 mg/kg, intraperitoneally) and the control group (5% glucose, intraperitoneally) in any of the tests. The oxaliplatin administration significantly reduced paw withdrawal threshold to mechanical stimuli and tail withdrawal latency to cold stimuli compared with the control group. A significant cold and mechanical allodynia was observed on day 1, which was more severe on day 2 and lasted for the 5 days of the experiment after oxaliplatin injection, compared with day 0 or the control group ( $P < 0.05$ ; **Figure 1A, B**). Exposure to a heat stimulus (42°C) did not display a significant difference for the oxaliplatin-treated group in heat allodynia compared with the control group ( $P > 0.05$ ; **Figure 1C**).

The effect of oxaliplatin treatment on the JAK/STAT3 signaling was measured by western blot assay. This showed that p-STAT3 was markedly increased on day 1, peaked on day 2 and lasted for 5 days after the injection of oxaliplatin. Compared with p-STAT3 protein expression on day 0, there were significant differences in the p-STAT3 expression from day 1 to day 5 ( $P < 0.05$ ; **Figure 2**). In addition, p-STAT3 immunoreactivity increased topographically in the spinal cord of rats induced by oxaliplatin treatment compared with the control group (**Figure 3**). Therefore, 2 days after a single injection of oxaliplatin (6 mg/kg, intraperitoneally) was chosen as the rat model of oxaliplatin-induced acute neuropathic pain for the following tests. Heat allodynia was not tested in the following experiments.

### AG490 administration alleviated cold and mechanical allodynia through inhibiting the activation of JAK/STAT3 signaling and depressing neuroinflammation in oxaliplatin-induced acute neuropathic pain of rats

In comparison with the oxaliplatin group, an intraperitoneal injection of AG490 clearly increased the cold and mechanical stimulus threshold in all three AG490 groups, but there was no significant difference among three AG490 groups (**Figure 4**).

Our results showed that p-STAT3 played an important

role in oxaliplatin-induced acute neuropathic pain, so we observed the effect of AG490 on the expression of p-STAT3 of spinal cord in oxaliplatin-treated rats. Western blot assay showed that AG490 treatment significantly attenuated the increased level of p-STAT3 protein, compared with the oxaliplatin group. There were no significant differences in p-STAT3 protein expression among the three AG490 groups (**Figure 5**). Moreover, the level of p-STAT3 immunoreactivity decreased topographically in the oxaliplatin + AG490 10 mg/kg group compared with the oxaliplatin group (**Figure 3**).

We also examined the effect of AG490 on the attenuation of neuroinflammation of oxaliplatin-induced acute neuropathic pain. The expression of IL-6 of spinal cord in rats was determined by immunohistochemical staining in the control, oxaliplatin and oxaliplatin + AG490 10 mg/kg groups. The expression of IL-6 in the oxaliplatin + AG490 10 mg/kg group was significantly reduced as compared with the oxaliplatin group (**Figure 6**).

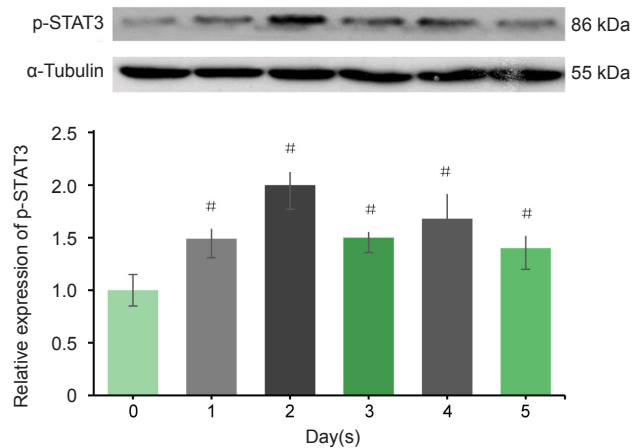
## Discussion

This study used a neuropathic pain model induced by a single intraperitoneal injection of 6 mg/kg oxaliplatin. The intraperitoneal injection route was selected based on the results of previous studies (Holmes et al., 1998; Jamieson et al., 2005; Ling et al., 2007; Li et al., 2015). In anticancer clinical trials, the oxaliplatin dosages used were 85 mg/m<sup>2</sup> every 2 weeks or 130 mg/m<sup>2</sup> every 3 weeks, and the maximum tolerated dose was 200 mg/m<sup>2</sup>, which corresponds approximately to 6 mg/kg (Raymond et al., 1998; Carrato et al., 2002). Hence, we considered this dose (6 mg/kg, intraperitoneally) was appropriate for use in our pain studies. Oxaliplatin-induced neuropathic pain represents a major obstacle to successful cancer treatment because of its prevalence in cases of individual as well as cumulative dosages. In this study, a single dose of oxaliplatin (6 mg/kg, intraperitoneally) significantly increased the cold and mechanical allodynia in rats. Significant cold and mechanical allodynia was observed on day 1, peaked on day 2, and lasted for 5 days after a single oxaliplatin injection. Simultaneously, p-STAT3 levels markedly increased on day 1, peaked on day 2, and lasted for 5 days. Therefore, we chose the 2 days post a single injection of oxaliplatin (6 mg/kg, intraperitoneally) to generate our rat model of oxaliplatin-induced neuropathic pain for subsequent experiments. There was no significant difference in heat allodynia between the oxaliplatin and control groups, so we excluded testing of heat allodynia in subsequent experiments.

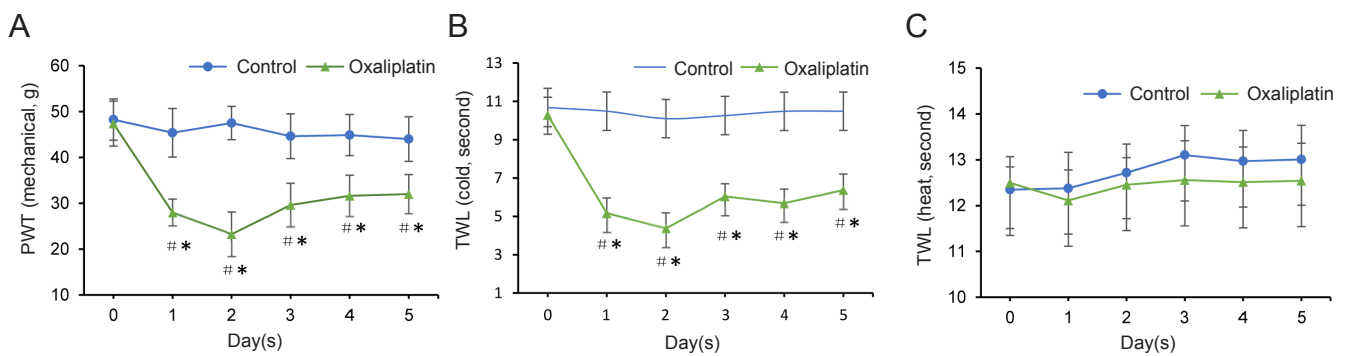
Tyrosine kinase inhibitors, including AG490, are effective for the treatment of various malignancies. Previous studies have demonstrated that administration of AG490 attenuates mechanical allodynia and/or thermal hyperalgesia in painful diabetic neuropathy, peripheral nerve injury, cyclophosphamide-induced bladder pain model and in  $\lambda$ -carrageenan-induced inflammatory pain (Dominguez et al., 2008; Cheppudira et al., 2009; Kou et al., 2013; Cheppudira et al., 2015). Our findings are in agreement with the findings of these studies. In our cold and mechanical allodynia tests, AG490 administration significantly increased the paw withdrawal threshold and tail withdrawal latency at all doses tested, but there was no dose-dependency among the three AG490 dose

groups. Future studies might investigate lower dosages to check for dose-dependency.

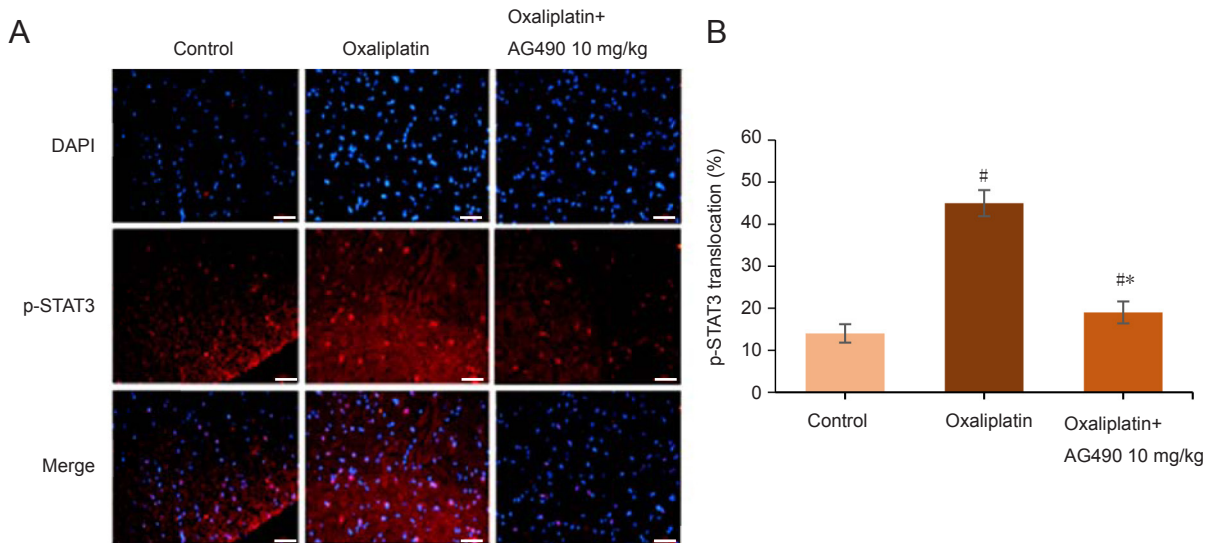
Some studies have observed that AG490 treatment reduces the levels of cytokine production, inflammatory cell infiltration and nitric acid production (Levitzki, 1990; Dimitrova and Ivanovska, 2008). Research on the possible mechanism underlying the protective effect of AG490 in oxaliplatin-induced acute neuropathic pain model of rats has mainly focused on the correlation between AG490 and the JAK/STAT3 signaling. Peripheral nerve injury evokes the activation of JAK/STAT3 with increased phosphorylation of STAT3, contributing to neuropathic pain (Yamauchi et al., 2006; Dominguez et al., 2008, 2010; Wang et al., 2014; Popiolek-Barczyk et al., 2015). Overexpression of p-STAT3 could be the mechanism that generates more severe pain behaviors in our oxaliplatin model rats. Spinal IL-33/ST2 signaling also aggravates neuropathic pain *via* activating the JAK/STAT3 signaling (Liu et al., 2015). Both AG490 and JAK/STAT3 inhibitor-1 induce less dorsal horn astrocyte proliferation and promote tactile allodynia recovery, in a study of rats with



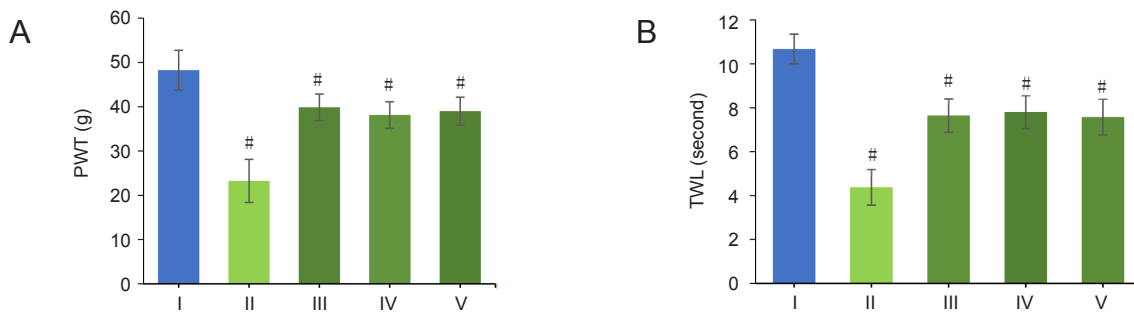
**Figure 2** Expression of p-STAT3 protein in spinal cord of oxaliplatin-treated (6 mg/kg, intraperitoneally) rats detected by western blot assay. Relative expression of p-STAT3 is expressed as the optical density ratio of p-STAT3/ $\alpha$ -tubulin. Data are represented as the mean  $\pm$  standard error of mean ( $n = 8$ ; one-way analysis of variance followed by the Tukey's multiple comparison test). # $P < 0.05$ , vs. day 0. p-STAT3: Phosphorylated STAT3.



**Figure 1** Influence of oxaliplatin on mechanical (A), cold (B), and heat (C) allodynia in rats. The behavioral tests were performed in control group (5% glucose, intraperitoneally), before (day 0) and after the administration of oxaliplatin (6 mg/kg, intraperitoneally) for 5 days. Data are expressed as the mean  $\pm$  standard error of mean ( $n = 8$ ; one-way analysis of variance followed by the Tukey's multiple comparison test). # $P < 0.05$ , vs. control group; \* $P < 0.05$ , vs. day 0. PWT: Paw withdrawal threshold; TWL: tail withdrawal latency.

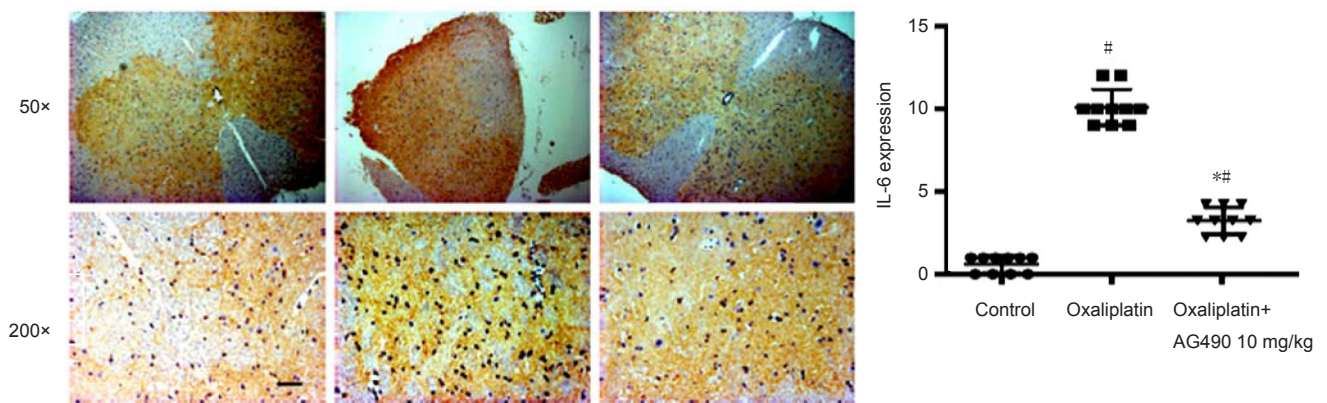


**Figure 3** Effect of Janus kinase inhibitor AG490 on the expression of p-STAT3 in the spinal cord tissue following oxaliplatin-induced acute neuropathic pain. (A) Immunofluorescence of p-STAT3 levels in the spinal cord sections of control, oxaliplatin (6 mg/kg oxaliplatin, intraperitoneally) and oxaliplatin (6 mg/kg oxaliplatin, intraperitoneally) + AG490 (AG490 10 mg/kg, intraperitoneally) groups. Scale bars: 20  $\mu$ m. (B) p-STAT3 translocation (%). Data are represented as the mean  $\pm$  standard error of mean ( $n = 8$ ; one-way analysis of variance followed by the Tukey's multiple comparison test). # $P < 0.05$ , vs. control group; \* $P < 0.05$ , vs. oxaliplatin group. DAPI: 4',6-Diamidino-2-phenylindole; p-STAT3: phosphorylated STAT3.



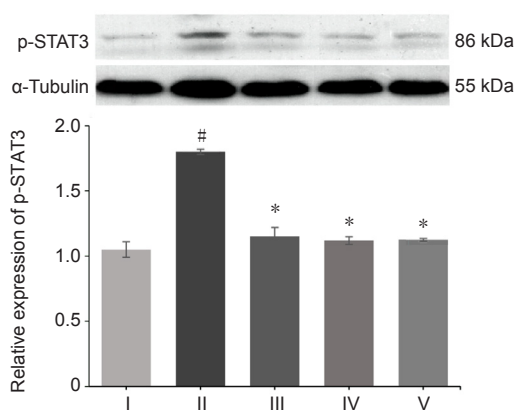
**Figure 4 Effects of AG490 on oxaliplatin-induced acute cold and mechanical allodynia in rats.**

The behavioral tests for mechanical (A) and cold (B) allodynia were performed in the control group (5% glucose, intraperitoneally), oxaliplatin (6 mg/kg, intraperitoneally) group and oxaliplatin (6 mg/kg, intraperitoneally) + AG490 (1, 5, 10 mg/kg, intraperitoneally) groups. Results are expressed as the mean  $\pm$  standard error of mean ( $n = 8$ ; one-way analysis of variance followed by the Tukey's multiple comparison test). # $P < 0.05$ , vs. control group. I: Control; II: Oxaliplatin; III: Oxaliplatin + AG490 1 mg/kg; IV: Oxaliplatin + AG490 5 mg/kg; V: Oxaliplatin + AG490 10 mg/kg. PWT: Paw withdrawal threshold; TWL: tail withdrawal latency.



**Figure 6 Expression of IL-6 in the spinal cord following oxaliplatin-induced acute neuropathic pain.**

Immunohistochemical (IHC) staining of IL-6 was performed on the spinal cord section in the control, oxaliplatin (6 mg/kg, intraperitoneally) and oxaliplatin (6 mg/kg, intraperitoneally) + AG490 (10 mg/kg, intraperitoneally) groups. Data are represented as the mean  $\pm$  standard error of mean ( $n = 8$ ; one-way analysis of variance followed by the Tukey's multiple comparison test); # $P < 0.05$ , vs. control group; \* $P < 0.05$ , vs. oxaliplatin group. Scale bar: 400  $\mu$ m. IL-6: Interleukin-6.



**Figure 5 Western blot assay of p-STAT3 expression in the spinal cord in each group.**

Relative expression of p-STAT3 is represented as the optical density ratio of p-STAT3/ $\alpha$ -tubulin in control, oxaliplatin (6 mg/kg, intraperitoneally) and oxaliplatin (6 mg/kg, intraperitoneally) + AG490 (1, 5, 10 mg/kg, intraperitoneally) groups. Data are expressed as the mean  $\pm$  standard error of mean ( $n = 8$ ; one-way analysis of variance followed by the Tukey's multiple comparison test). # $P < 0.05$ , vs. control group; \* $P < 0.05$ , vs. oxaliplatin group. I: Control; II: Oxaliplatin; III: Oxaliplatin + AG490 1 mg/kg; IV: Oxaliplatin + AG490 5 mg/kg; V: Oxaliplatin + AG490 10 mg/kg. p-STAT3: Phosphorylated signal transducer and activator of transcription 3.

spinal nerve injury (Tsuda et al., 2011). Another promising agent for treating neuropathic pain, WP1066, is an inhibitor of STAT3 signaling and relieves the pain behavior of rats with chronic constriction injury (Xue et al., 2014). All these references suggest that JAK/STAT3 signaling is activated in both peripheral and spinal nerve injury, while its inhibition alleviates neuropathic pain. Similarly, in our study, p-STAT3 protein was overexpressed in rats with oxaliplatin-induced acute neuropathic pain. Our experimental findings indicated that STAT3 was significantly phosphorylated from day 1 to day 5 after a single oxaliplatin treatment, peaking on day 2. These data suggested that JAK/STAT3 signaling was activated in the spinal cord after a single oxaliplatin injection. AG490 treatment inhibited STAT3 phosphorylation in the spinal cord of rats with oxaliplatin-induced acute neuropathic pain.

In models of neuropathic pain, the time course of the expression of IL-6 has been well characterized in the peripheral and central nervous systems (Rutkowski and DeLeo, 2002; Lee et al., 2004; Marchand et al., 2005; Sacerdote et al., 2013). Up-regulation of mRNA transcripts and immunoreactivity for the proinflammatory mediator of IL-6 have also been observed in the spinal cord following nerve injury (Raghavendra et al.,

2003; Sacerdote et al., 2013). Therefore, we determined to see whether AG490 could inhibit spinal IL-6 expression in our rat model. We found that the spinal cord showed increased expression of IL-6 after oxaliplatin treatment, which was down-regulated by AG490 treatment. These results suggest that the effect of AG490 is probably anti-neuroinflammatory.

This study has revealed a new role and indicated a potential application for AG490 in attenuating oxaliplatin-induced acute neuropathic pain. AG490 prevents oxaliplatin-induced acute neuropathic pain from becoming chronic neuropathic pain, allowing the continuation of oxaliplatin therapy. This study has provided significant experimental and theoretical background for further clinical study. In conclusion, AG490 is an effective way in reducing oxaliplatin-induced acute neuropathic pain. This new function of AG490 is anti-neuroinflammatory and probably acts by inhibiting the JAK/STAT3 signaling; however, the detailed and precise mechanism still needs further investigation.

**Author contributions:** SFL and YPW conceived and designed the study. XZ and BSO performed the experiments. SFL analyzed the data and wrote the paper. XZ provided reagents/materials/analysis tools. SFL wrote the paper. All authors approved the final version of the paper.

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**Institutional review board statement:** The study protocol was approved by the Institutional Animal Care and Use Committee of Xiangya Research Laboratory, China (approval No. IACUC-2016-0157). The experimental procedure followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1985).

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## References

- Argyriou AA, Cavaletti G, Briani C, Velasco R, Bruna J, Campagnolo M, Alberti P, Bergamo F, Cortinovis D, Cazzaniga M, Santos C, Papadimitriou K, Kalofonos HP (2013) Clinical pattern and associations of oxaliplatin acute neurotoxicity: a prospective study in 170 patients with colorectal cancer. *Cancer* 119:438-444.
- Carrato A, Gallego J, Diaz-Rubio E (2002) Oxaliplatin: results in colorectal carcinoma. *Crit Rev Oncol Hematol* 44:29-44.
- Cheppudira BP, Garza TH, Petz LN, Clifford JL, Fowler M (2015) Anti-hyperalgesic effects of AG490, a Janus kinase inhibitor, in a rat model of inflammatory pain. *Biomed Rep* 3:703-706.
- Cheppudira BP, Girard BM, Malley SE, Dattilio A, Schutz KC, May V, Vizzard MA (2009) Involvement of JAK-STAT signaling/function after cyclophosphamide-induced bladder inflammation in female rats. *Am J Physiol Renal Physiol* 297:F1038-1044.
- Dimitrova P, Ivanovska N (2008) Tyrosinase AG-490 inhibited the acute phase of zymosan-induced inflammation. *Int Immunopharmacol* 8:1567-1577.
- Dominguez E, Rivat C, Pommier B, Mauborgne A, Pohl M (2008) JAK/STAT3 pathway is activated in spinal cord microglia after peripheral nerve injury and contributes to neuropathic pain development in rat. *J Neurochem* 107:50-60.
- Dominguez E, Mauborgne A, Mallet J, Desclaux M, Pohl M (2010) SOCS3-mediated blockade of JAK/STAT3 signaling pathway reveals its major contribution to spinal cord neuroinflammation and mechanical allodynia after peripheral nerve injury. *J Neurosci* 30:5754-5766.
- Holmes J, Stanko J, Varchenko M, Ding H, Madden VJ, Bagnell CR, Wyrick SD, Chaney SG (1998) Comparative neurotoxicity of oxaliplatin, cisplatin, and ormaplatin in a Wistar rat model. *Toxicol Sci* 46:342-351.
- Inoue K, Nagai S, Kaneko S, Uchida S, Hayashi Y, Higuchi T, Yoshida T, Takei H, Tabei T (2011) Efficacy of oxycodone against anti-cancer agent-induced pain in breast cancer patients during adjuvant treatment before and after surgery. *Gan To Kagaku Ryoho* 38:1137-1142.
- Jamieson SM, Liu J, Connor B, McKeage MJ (2005) Oxaliplatin causes selective atrophy of a subpopulation of dorsal root ganglion neurons without inducing cell loss. *Cancer Chemother Pharmacol* 56:391-399.
- Kou ZZ, Li CY, Tang J, Hu JC, Qu J, Liao YH, Wu SX, Li H, Li YQ (2013) Down-regulation of insulin signaling is involved in painful diabetic neuropathy in type 2 diabetes. *Pain Physician* 16:E71-83.
- Lee HL, Lee KM, Son SJ, Hwang SH, Cho HJ (2004) Temporal expression of cytokines and their receptors mRNAs in a neuropathic pain model. *Neuroreport* 15:2807-2811.
- Levitzi A (1990) Tyrosinase--potential antiproliferative agents and novel molecular tools. *Biochem Pharmacol* 40:913-918.
- Li D, Lee Y, Kim W, Lee K, Bae H, Kim SK (2015) Analgesic effects of bee venom derived phospholipase A(2) in a mouse model of oxaliplatin-induced neuropathic pain. *Toxins (Basel)* 7:2422-2434.
- Lim BS, Moon HJ, Li DX, Gil M, Min JK, Lee G, Bae H, Kim SK, Min BI (2013) Effect of bee venom acupuncture on oxaliplatin-induced cold allodynia in rats. *Evid Based Complement Alternat Med* 2013:369324.
- Ling B, Coudore-Civiale MA, Balayssac D, Eschaliere A, Coudore F, Authier N (2007) Behavioral and immunohistological assessment of painful neuropathy induced by a single oxaliplatin injection in the rat. *Toxicology* 234:176-184.
- Liu S, Mi WL, Li Q, Zhang MT, Han P, Hu S, Mao-Ying QL, Wang YQ (2015) Spinal IL-33/ST2 signaling contributes to neuropathic pain via neuronal CaMKII-CREB and astroglial JAK2-STAT3 cascades in mice. *Anesthesiology* 123:1154-1169.
- Marchand F, Perretti M, McMahon SB (2005) Role of the immune system in chronic pain. *Nat Rev Neurosci* 6:521-532.
- Nagashima M, Ooshiro M, Moriyama A, Sugishita Y, Kadoya K, Sato A, Kitahara T, Takagi R, Urita T, Yoshida Y, Tanaka H, Oshiro T, Okazumi S, Katoh R (2014) Efficacy and tolerability of controlled-release oxycodone for oxaliplatin-induced peripheral neuropathy and the extension of FOLF-FOX therapy in advanced colorectal cancer patients. *Support Care Cancer* 22:1579-1584.
- Popielek-Barczyk K, Kolosowska N, Piotrowska A, Makuch W, Rojewska E, Jurga AM, Pilat D, Mika J (2015) Parthenolide relieves pain and promotes M2 microglia/macrophage polarization in rat model of neuropathy. *Neural Plast* 2015:676473.
- Raghavendra V, Tanga F, DeLeo JA (2003) Inhibition of microglial activation attenuates the development but not existing hypersensitivity in a rat model of neuropathy. *J Pharmacol Exp Ther* 306:624-630.
- Raymond E, Chaney SG, Taamma A, Cvitkovic E (1998) Oxaliplatin: a review of preclinical and clinical studies. *Ann Oncol* 9:1053-1071.
- Rutkowski MD, DeLeo JA (2002) The role of cytokines in the initiation and maintenance of chronic pain. *Drug News Perspect* 15:626-632.
- Sacerdote P, Franchi S, Moretti S, Castelli M, Proccacci P, Magnaghi V, Panerai AE (2013) Cytokine modulation is necessary for efficacious treatment of experimental neuropathic pain. *J Neuroimmunol Pharmacol* 8:202-211.
- Tsuda M, Kohro Y, Yano T, Tsujikawa T, Kitano J, Tozaki-Saitoh H, Koyanagi S, Ohdo S, Ji RR, Salter MW, Inoue K (2011) JAK-STAT3 pathway regulates spinal astrocyte proliferation and neuropathic pain maintenance in rats. *Brain* 134:1127-1139.
- Wang N, Liang H, Zen K (2014) Molecular mechanisms that influence the macrophage m1-m2 polarization balance. *Front Immunol* 5:614.
- Xue ZJ, Shen L, Wang ZY, Hui SY, Huang YG, Ma C (2014) STAT3 inhibitor WP1066 as a novel therapeutic agent for bCCI neuropathic pain rats. *Brain Res* 1583:79-88.
- Yamauchi K, Osuka K, Takayasu M, Usuda N, Nakazawa A, Nakahara N, Yoshida M, Aoshima C, Hara M, Yoshida J (2006) Activation of JAK/STAT signalling in neurons following spinal cord injury in mice. *J Neurochem* 96:1060-1070.

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