

Inhibitory Effects of Valproic Acid in Oxaliplatin-Induced Neuropathy in Rat Model

Ke Ren, Kai Yi, Yidan Dai, Motoi Fujiwara, Tetsuo Ohta, Hayato Terayama, Kou Sakabe, Shuang-Qin Yi

Ke Ren, Yidan Dai, Motoi Fujiwara, Shuang-Qin Yi, Department of Frontier Health Sciences, Graduate School of Human Health Sciences, Tokyo Metropolitan University, Tokyo, Japan

Kai Yi, Department of Anatomy, Tokyo Medical University Graduate School of Medicine, Tokyo, Japan

Tetsuo Ohta, Deptment of Gastroenterological Surgery, Kanazawa University, Kanazawa, Japan

Hayato Terayama, Kou Sakabe, Deptment of Anatomy, Tokai University Graduate School of Medicine, Isehara, Japan

Conflict-of-interest statement: The authors declare that there is no conflict of interest regarding the publication of this paper.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Correspondence to: Shuang-Qin Yi, MD, PhD, Department of Frontier Health Sciences, Graduate School of Human Health Sciences, Tokyo Metropolitan University, Tokyo, Japan.

Email: yittmniu@tmu.ac.jp

Telephone: +81-3-3819-1211

Ke Ren Kai Yi, are co-contributors for this study and this paper.

Kai Yi is working in Wuhan Institute of Bioengineering now, Wuhan, China.

Received: October 11, 2017

Revised: December 5, 2017

Accepted: December 7, 2017

Published online: December 21, 2017

ABSTRACT

AIM: Oxaliplatin is a third-generation platinum-based chemotherapy drug, introduced for management of the advanced stages of metastatic colorectal cancer. However, repeated administration of oxaliplatin induced acute and chronic peripheral neuropathy. Valproic acid (VPA)

is a neurotherapeutic drug used widespread and worldwide as therapy for seizures, bipolar disorder, and migraine, including children, adult and women of reproductive age.

MATERIALS AND METHODS: In the present study, we investigated the effect of VPA in prevention of oxaliplatin-induced peripheral neuropathy in the rat model. We demonstrated that VPA (300 mg/kg) relieved the oxaliplatin (4mg/kg)-induced peripheral neuropathy using behavioral tests, biochemical tests, and histopathological and immunohistochemical evaluations.

RESULTS: VPA administration significantly attenuated the mechanical hyperalgesia by oxaliplatin-induced in rats. VPA exerted a significant protective effect by reducing the occurrence of multinucleolated neurons and the nucleolar eccentricity caused on lumbar dorsal root ganglion from oxaliplatin-treated rats. It revealed an inhibitory effect of VPA on the number and activation of microglia and astrocytes in the dorsal horn of the spinal cord. However, VPA was unable to prevent demyelination and degeneration of nerve fibers from oxaliplatin-induced peripheral neurotoxicity.

COLUSION: The present results demonstrated for the first time that VPA administration ameliorated the oxaliplatin-induced behavioral, biochemical and histopathological changes in rats. The VPA-mediated effects in this study may be attributed to neuroprotection properties and ameliorating oxaliplatin-induced astrocytes and microglial activation. VPA may offer a dual protective approach against etiological factors and resulting maladaptative plasticity.

Key words: Oxaliplatin; Peripheral neuropathy; Mechanical allodynia; Valproic acid; Astrocyte and Microglia; Nucleolar eccentricity

© 2017 The Author(s). Published by ACT Publishing Group Ltd. All rights reserved.

Ren K, Yi K, Dai Y, Fujiwara M, Ohta T, Terayama H, Sakabe K, Yi SQ. Inhibitory Effects of Valproic Acid in Oxaliplatin-Induced Neuropathy in Rat Model. *Journal of Gastroenterology and Hepatology Research* 2017; **6(6)**: 2461-2469 Available from: URL: <http://www.ghrnet.org/index.php/joghr/article/view/2157>

INTRODUCTION

Oxaliplatin is a third-generation platinum-based chemotherapy drug in various solid tumours, in particular, it was introduced for the

management of the advanced stages of metastatic colorectal cancer^[1]. However, it has been reported that repeated administration of oxaliplatin induced severe acute and chronic peripheral neuropathy^[2]. Oxaliplatin induced neuropathy can persist from months to years beyond chemotherapy completion, causing significant challenges for cancer survivors due to negative influence on function and quality of life. Oxaliplatin neurotoxicity resulted in chemotherapy dose reductions or early discontinuation^[3].

Acute neuropathy shows cold hyperalgesia in the early phase and includes acral paresthesias enhanced by exposure to cold in 71 to 95% of all patients^[4,5]. It has been thought that the acute neuropathy is not due to morphological damage of the nerve^[6] and is due to alternations of voltage-gated Ca²⁺ and K⁺ channels^[7]. On the other hand, the dose-limiting toxicity of this compound is the development of peripheral neuropathy with glove-and-stocking distribution sensory loss, combined with paresthesia, dysesthesia, pain, and motor neuropathy^[3,6,8]. A chronic neurological syndrome, related to the total cumulative dose as well as the dose-intensity of treatment persists between and after treatments^[9] negatively influencing patient's quality of life. Thus, these neuropathies are a major clinical problem in oxaliplatin chemotherapy.

To ameliorate oxaliplatin-induced neuropathy, various treatments by animal experiments have been suggested including gabapentin^[10], neurotrophin^[6], carbamazepine^[11], phosphatidylcholine^[12], N-palmitoylethanolamine^[3], exenatide^[8], and goshajinkigan^[2]. There is no currently univocally-accepted proven therapy for oxaliplatin-induced neuropathy. Most randomized controlled trials testing a variety of drugs with diverse mechanisms of action failed to reveal an effective treatment. Recently, most reports including glutathione^[13], duloxetine^[14], Vitamin E^[15], oxycodone^[16], goshajinkigan^[17], pregabalin^[18] and MR309 (a novel selective sigma-1 receptor ligand previously developed as E-52862)^[19] show some effects. Neuroprotective, safe, preventive agents as adjuvant to chemotherapy are a therapeutic need.

Valproic acid (VPA) is a neurotherapeutic drug prescribed worldwide as therapy for seizures, bipolar disorder, and migraine, including children, adult and women of reproductive age. It is one of the major antiepileptic drugs in clinical practice and the drug of choice par excellence for all varieties of generalised epilepsy syndromes, primary or symptomatic^[20]. Recently, VPA exerts protective effects for various neurological diseases, including spinal cord injury^[21,22], stroke^[23], traumatic brain injury^[24], motor neuron diseases^[25], Parkinson's disease^[26], Alzheimer's disease^[27] and Huntington's disease^[28]. There is now accumulating evidence that VPA may have potential in the treatment of central nervous system disorders and the neuroprotective functions are linking with its inhibition on histone deacetylases (HDAC)^[29,30]. It has recently been demonstrated that VPA robustly promotes neurite outgrowth, activates the extracellular signal regulated kinase pathway^[30, 31,32]. However, the effect of VPA on the oxaliplatin-induced neuropathy remains unexplored.

Accordingly, in the present study, we investigated the effect of VPA in prevention of oxaliplatin induced periphery neuropathy in the rat model. The anti-neuropathic role of VPA was evaluated in oxaliplatin-treated animals by analyzing pain behavior in relation to morphological and functional protection of the nervous system.

MATERIALS AND METHODS

1. Animals

Six-week-old male Sprague-Dawley rats weighing 200–250 g (Japan

SLC, Shizuoka, Japan) were employed in the present study. Animals were housed in groups of three to four per cage (size 26 × 41 cm), fed a standard laboratory diet and tap water ad libitum, and kept at 25 ± 2°C with a 12 h light/dark cycle, light at 8 a.m. All experiments were approved by the Experimental Animal Care and Use Committee of Tokyo Metropolitan University according to the National Institutes of Health guidelines (Permit Number: A28-16, A29-8), and we followed International Association for the Study of Pain (IASP) Committee for Research and Ethical Issues guidelines for animal research^[33]. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2. Oxaliplatin model and pharmacological treatments

Oxaliplatin (Elplat®) was obtained from Yakult Co., Ltd. (Tokyo, Japan). VPA was purchased from Nippon Zoki Pharmaceutical Co. (Osaka, Japan).

The rats ($n = 18$) were divided into three groups (6 rats in each group) and treated as follows. *Oxaliplatin group*: oxaliplatin (4 mg/kg) was injected in intraperitoneal injection (i.p.) in volumes of 10 ml/kg twice weekly for 4 weeks (Days 1, 2, 8, 9, 15, 16, 22 and 23) (Figure 1). Oxaliplatin was dissolved in a 5% glucose-water solution. The dose of oxaliplatin followed previous reports^[6,34]. *Oxaliplatin + VPA group*: administration both oxaliplatin (as above) and VPA (300 mg/kg) daily twice a day for 4 weeks in i.p. The dose of VPA followed previous reports^[35,36]. *Control group*: injection of vehicle (5% glucose solution) instead of oxaliplatin and VPA.

3. Assessment of general toxicity

The measurement of the body weights of rats was performed on Days 0, 1, 2, 3, 8, 9, 10, 15, 16, 17, 22, 23, 24 and 28 in every groups, including on the day of treatment and immediately prior to sacrifice. Rats were examined daily for abnormal clinical signs such as piloerection, hindlimb weakness, gait disturbance or gastrointestinal disorders such as diarrhea.

4. von Frey test for mechanical allodynia

The mechanical allodynia was assessed by von Frey test. The von Frey test was performed before the first drug administration (on Day 0) and on Days 5, 15, 21 and 28 (Figure 1). On Days 5, 15 and 21, the test was performed before drug administration. Rats were placed in a clear plastic box (20 × 17 × 13 cm) with a wire mesh floor and allowed to habituate for 30 min prior to testing. von Frey filaments (Aesthesio®, Precise Tactile Sensory Evaluator 20 pieces Kit with Carrying case, USA) ranging 1-15 g bending force were applied to the midplantar skin of each hind paw with each application held for 6 s. Fifty percent paw withdrawal thresholds were determined by a modification of up-down method that described by Kawashiri *et al*^[6] and Ushio *et al*^[37]. First, each hind paw was touched with some filaments from 1 g up to the force that rat exhibited the withdrawal response, in ascending order. Next, the paw was touched with some filaments from 15 g down to the force that rat did not exhibit the response, in descending order. These up and down steps were repeated three times. Fifty percent thresholds were determined by average of the weakest force in each up or down step.

5. Acetone test for cold hyperalgesia

The cold hyperalgesia was assessed by acetone test. The acetone test was performed before the first drug administration and on the first day of drug administration (on Day 0 and Day 1) and on Days 5, 7, 14, 21 and 28 (Figure 1) according to the method described by Kawashiri *et al*^[6] and Ushio *et al*^[37]. On Days 1, 5, 7, 14 and 21,

test was performed before drug administration. Rats were placed in a clear plastic box (20 × 17 × 13 cm) with a wire mesh floor and allowed to habituate for 30 min prior to testing. Fifty microlitre of acetone (Wako Pure Chemical Ltd., #016-00346, Osaka, Japan) was sprayed onto the plantar skin of each hind paw three times with a micro sprayer, and rats were observed for 40 s from the start of the acetone spray. The number of elevation of each hind paw was recorded.

6. Assay of sciatic nerve axonal degeneration

On Day 28, the rats were deeply anesthetized with pentobarbital (50 mg/kg), and transcardially perfused with phosphate-buffered saline (PBS) (0.1M, pH 7.4), followed by 2.5% (w/v) glutaraldehyde in PBS. The sciatic nerves were rapidly dissected, and the samples were kept overnight in the same fixative at 4 °C. The fixed fibers were post-fixed with 1% osmium tetroxide solution for 3 hours, dehydrated in a graded alcohol series, and embedded in EPON 815 (Wako, Japan). For light microscopy, semi-thin sections were cut from each block and stained with toluidine blue. The stained sections were observed using a light microscope (BX63, Olympus Corp., Tokyo, Japan). The density of axon area was calculated by image analysis software (ImageJ 1.50a; Wayne Rasband, National Institutes of Health, MD, USA).

7. Histopathological assessment on dorsal root ganglia

On Day 28, the dorsal root ganglion (DRGs) and spinal cord specimens (at segments L4 and L5) were excised from rats of each group, and fixed by immersion in 4% PFA overnight at 4°C. The tissues were then washed with PBS, dehydrated with ascending grades of reagent alcohol, cleared in two changes of xylene,

infiltrated with paraffin, and sliced to 5 µm, mounted on charged slides. The DRG specimens were stained with Azan-Mallory method as manual, and the spinal cord specimens were performed with immunohistochemistry for GFAP and Iba1 as below 2.8.

Cellular dimensions of L4-L5 DRGs were measured using a method adapted from Di Cesare Mannelli *et al.*^[3,38]. In these sections, using a 40x objective lens, the numbers of neurons with nuclei, nucleoli, multiple nucleoli, and nucleolar eccentricity (eccentric nucleolus) were counted. The nucleolus were considered eccentric when its center (or that of the largest one if there appeared to be more than one) lay in the outer half of the radius of the nucleus. The results were expressed as percentage of those cells with a visible nucleolus. Four consecutive sections for each animal were analyzed. The reported data were obtained by averaging the data of L4 and L5 ganglia.

8. Immunohistochemical evaluation of GFAP and Iba1 in L4-L5 spinal cord

The immunohistochemical procedures were performed according to our previous study^[39]. Briefly, after rinsing the fixed tissue specimens in 0.01 M PBS (pH 7.4), endogenous peroxidase activity was inhibited by 30-min incubation in methanol containing 0.3% (v/v) hydrogen peroxide. After rinsing in PBS, the sections were blocked with normal goat serum for 1 h at room temperature, were then incubated for overnight at 4°C in PBS containing the primary antibodies, against glial fibrillary acidic protein (GFAP; mouse, 1:300; MAB3402, Chemicon, Temecula, USA) for astrocyte staining and Iba1 (rabbit, 1:200; #019-19741, Wako Pure Chemicals, Osaka, Japan) for microglial staining. After rinsing in PBS, sections were incubated in donkey anti-mouse IgG secondary antibody labeled with Alexa Fluor

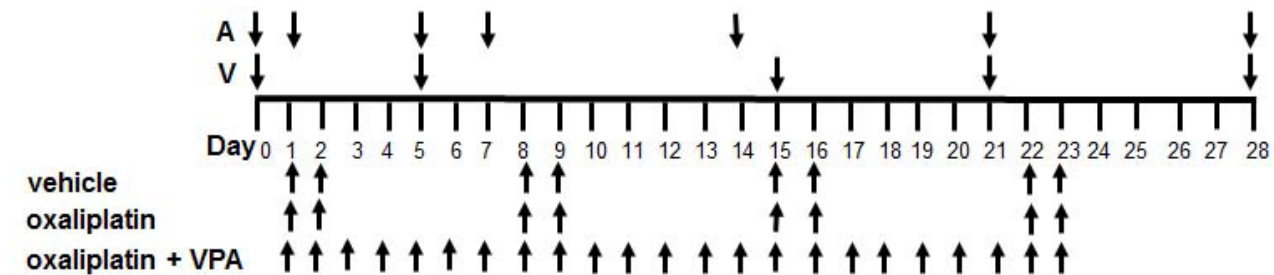


Figure 1 Schematic of drug administration and behavioral test. A: Acetone test; V: von Frey test; vehicle: intraperitoneal injection (i.p.) with 5% glucose solution; oxaliplatin: oxaliplatin 4mg/kg i.p.; oxaliplatin + VPA group: oxaliplatin 4mg/kg and VPA 300mg/kg i.p., respectively.

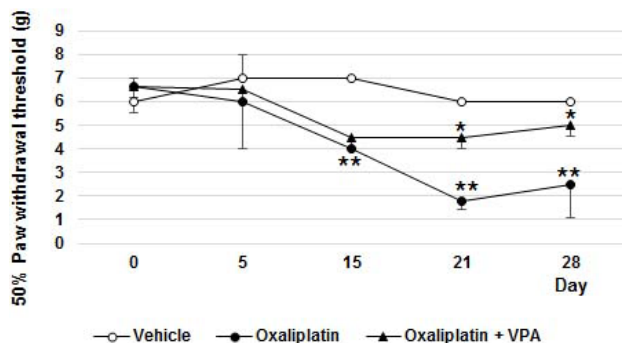


Figure 2 Effects of repeated administration of VPA on oxaliplatin-induced mechanical allodynia in von Frey test in rats. Oxaliplatin (4 mg/kg) was administered i.p. twice a week for 4 weeks. VPA (300 mg/kg) was administered daily twice a day for 4 weeks. The von Frey test was performed before the first drug administration (on day 0) and on days 5, 15, 21 and 28. Values were expressed as the mean ± standard error mean of 6 animals. ** $P < 0.01$ compared with vehicle. * $P < 0.05$ compared with oxaliplatin alone.

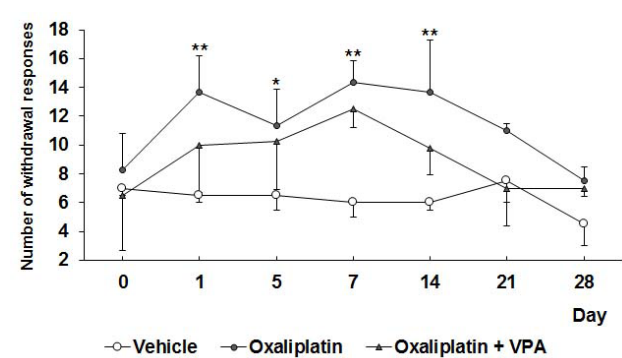


Figure 3 Effects of repeated administration of VPA on oxaliplatin-induced cold hyperalgesia in acetone test in rats. Oxaliplatin (4 mg/kg) was administered i.p. twice a week for 4 weeks. VPA (300 mg/kg) was administered daily twice a day for 4 weeks. The acetone test was performed before the first drug administration (on day 0) and on days 1, 3, 5, 7, 14, 21 and 28. Values are expressed as the mean ± standard error mean of 6 animals. * $P < 0.05$, ** $P < 0.01$ compared with vehicle.

488 (1:2000, Thermo Fisher Scientific, Rockford, USA) and chicken anti-rabbit IgG secondary antibody labeled with Alexa Fluor 488 (1:500, Thermo Fisher Scientific, Rockford, USA), respectively, at room temperature for 1 h.

Negative control sections (no exposure to the primary antisera) were processed concurrently with the other sections for all immunohistochemical studies. We obtained a single optical density value for the dorsal horns by averaging the two sides in each rat, and these values were compared to the homologous average values from the vehicle-treated animals.

9. Quantitative analyses of GFAP and Iba1 immunohistochemistry

Images were acquired by a motorized ZEISS ImagerM1 microscope equipped with a DS-Fi3 camera (Nikon, Tokyo, Japan). Morphological examination of astrocyte and microglia morphology was assessed by inspection of at least three fields (20 x) in the dorsal horn per section.

Quantitative analysis of GFAP and Iba1-positive cells was performed by collecting at least three independent fields through a 20 X 0.5NA objective. The densities of GFAP and Iba1-positive cells were calculated by means of the automatic thresholding and segmentation features of ImageJ (ImageJ 1.50a; Wayne Rasband, National Institutes of Health, MD, USA). Results, given as the area fraction (%) occupied by the thresholded GFAP- or Iba1-positive cell number, respectively. Five spinal cord sections were analyzed for each animal.

10. Statistical analyses

Data are expressed as the mean \pm standard deviation (SD). ANOVA and the Tukey's multiple comparison tests were employed for statisti-

cal analysis. All tests were performed as two-sided test and a *p* value of < 0.05 was accepted as significant.

RESULTS

1. General toxicity of oxaliplatin

Rats were injected with either oxaliplatin (4 mg/kg) or VPA (300 mg/kg) at dosages corresponding to human chemotherapy, while 5% glucose solution was used as a control treatment (Figure 1). No deterioration in general status was observed in any of the groups, and no rats died in the course of our experiments. No significant differences in body weight were observed between groups at any time (data not shown).

2. Effects of VPA on mechanical allodynia in oxaliplatin-induced neuropathy

Before the first oxaliplatin injection, there were no significant differences in withdrawal thresholds in all groups in the von Frey test. Oxaliplatin significantly reduced the withdrawal threshold compared with vehicle on Days 15, 21 and 28 ($P < 0.01$). Repeated administration of VPA significantly inhibited the oxaliplatin-induced reduction of the withdrawal threshold on Days 21 and 28 ($P < 0.05$) (Figure 2).

3. Effects of VPA on cold hyperalgesia in oxaliplatin-induced neuropathy

In the acetone test, there were no significant differences in number of withdrawal responses in all groups before the first oxaliplatin injection. Oxaliplatin significantly increased the number of withdrawal responses compared with vehicle on Days 1, 5, 7 and 14 ($P < 0.05$ or 0.01). However, no significant difference in withdrawal responses

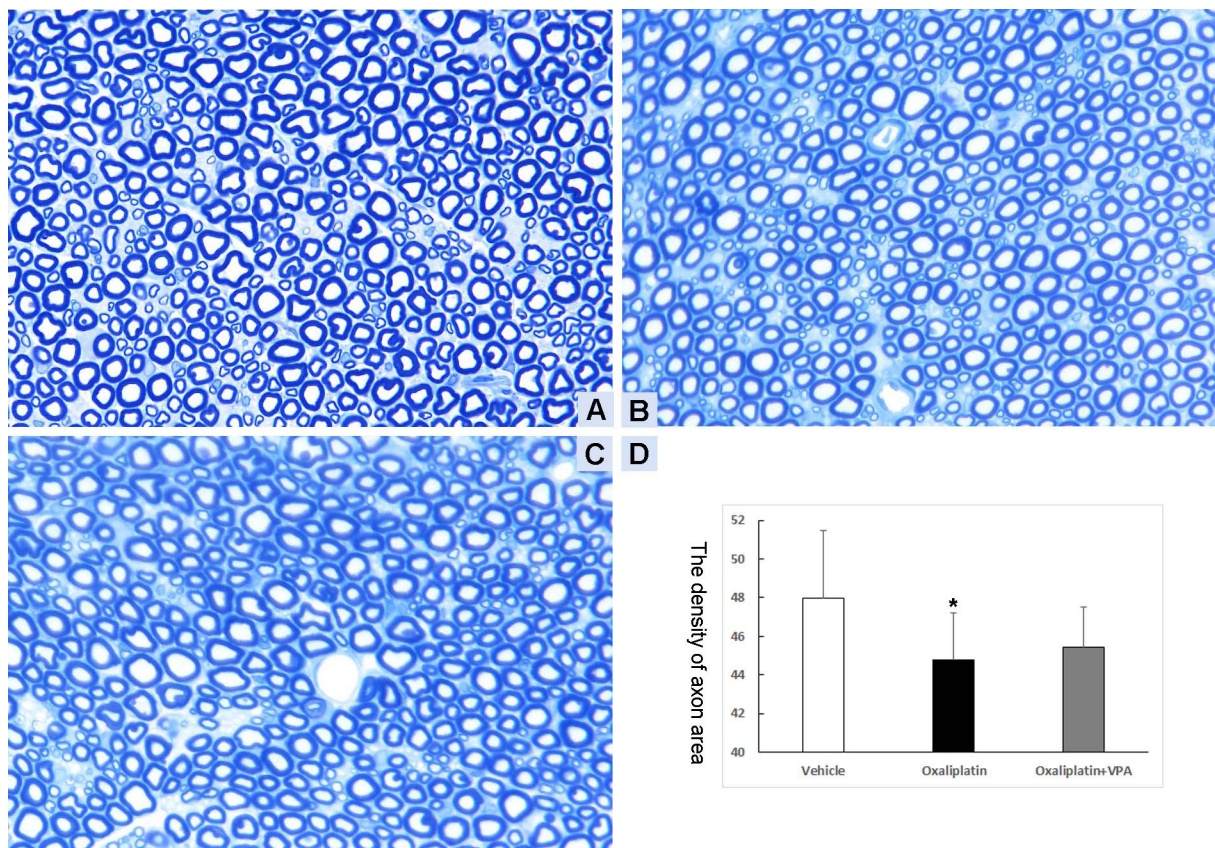


Figure 4 Effect of repeated administration of VPA on histological change induced by oxaliplatin (B) in rat sciatic nerve. On day 28, The sciatic nerve was harvested, and samples were stained with toluidine blue. Images were captured at 800 \times magnification. The area of axon was calculated by image analysis software (ImageJ 1.50a). Values were expressed as the mean \pm standard error mean of six animals. * $P < 0.05$ compared with vehicle (A). (C), oxiliplatin + VPA.

compared to the vehicle group was observed on Days 21 and 28 ($P > 0.05$). Repeated administration of VPA did not significantly inhibit the oxaliplatin-induced increase of the number of withdrawal responses on any days ($P > 0.05$) (Figure 3).

4. Effect of VPA on oxaliplatin-induced histological change in rat sciatic nerve

Histological abnormalities in sciatic nerve were observed in vehicle-, oxaliplatin-treated and oxaliplatin + VPA-treated rats on Day 28. The quantification analysis showed that oxaliplatin caused the decrease in the density of myelinated fibers and the degeneration of myelinated fibers in rat sciatic nerve ($P < 0.05$, Figure 4B), and co-treatment with VPA had no effect on the oxaliplatin-induced decrease of the density of myelinated fibers ($P > 0.05$, Figure 4C).

5. Effect of VPA on morphological derangement of DRG neurons

Morphologic and morphometric determinations on morphological derangement of DRG neurons were performed under the light microscope after Azan–Mallory stain. On day 28, oxaliplatin-induced damage was evidenced by the occurrence of multinucleolated neurons (Figure 5B) and nucleolar eccentricity (Figure 5B and C) distributed on small, medium and large neurons. VPA exerted a significant protective effect by reducing the occurrence of eccentric nucleolus neurons caused by oxaliplatin (Figure 5D).

6. Effect of VPA treatment on glial cell activation profile in the spinal cord

To establish a relationship between pain relief and glial modulation the cell densities of astrocytes and microglia were calculated in the dorsal horn of the spinal cord using immunohistochemistry with antibodies against GFAP and Iba1, respectively.

In the spinal cord, repeated oxaliplatin injections (Day 28) induced an increase in GFAP-positive cells (Figure 6B), astrocyte density increased over the entire surface of the spinal cord, particularly in the superficial laminae. VPA treatment prevented the increase in the density of the dorsal horn GFAP-positive cells (Figure 6C).

The same as GFAP and shown in Figure 7B, the number of Iba1-expressing cells in dorsal horn superficial laminae of oxaliplatin-treated rats was significantly increase than the vehicle group. VPA treatment prevented the increase in the density of the dorsal horn microglial cells (Figure 7C).

DISCUSSION

In the present study, to demonstrate that VPA-treatment relieved the oxaliplatin-induced peripheral neuropathy, according to previous studies^[10,12], oxaliplatin (4 mg/kg) was injected intraperitoneally to rats twice a week, and VPA (300 mg/kg) was administered daily twice a day for 4 weeks. The results were analysed using behavioral tests, and histopathological or immunohistochemical evaluations. In

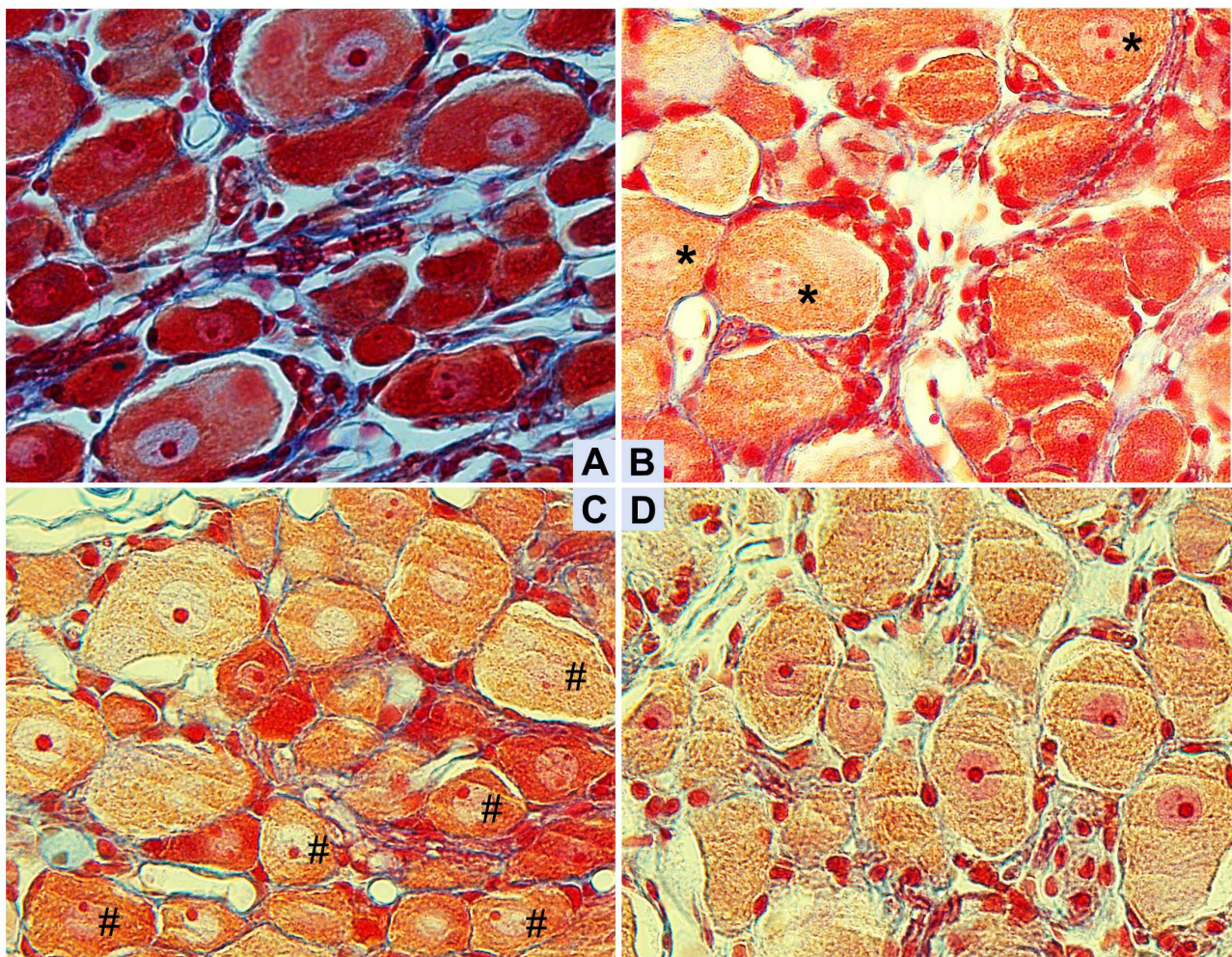


Figure 5 Morphological aspects of the peripheral nervous system. The protective effect of repeated administrations of VPA was evaluated on oxaliplatin-damaged DRGs on day 28. DRG sections were stained by the Azan–Mallory method. Light micrographs (original magnification 200 x) were analyzed by counting the incidence of eccentric nucleoli (#) and multinucleolated neurons (*).

the von Frey test, VPA administration significantly attenuated the mechanical hyperalgesia induced by oxaliplatin injection. On the other hand, it was almost ineffective against the oxaliplatin-induced cold hyperalgesia in the acetone test. Therefore, it suggested that VPA has a protective effect on oxaliplatin-induced chronic peripheral neuropathy on the mechanical hyperalgesia.

VPA as a broad-spectrum HDAC inhibitor, is an anticonvulsant and mood-stabilizing drug with neuroprotective effects^[40]. The HDAC inhibitors, such as trichostatin A and valproic acid, restored peripheral and systemic morphine analgesia in neuropathic pain. It suggests that HDAC inhibitors could serve as adjuvant analgesics to morphine for the management of neuropathic pain^[35]. A clinical investigation demonstrated sodium valproate is well-tolerated, and provides significant subjective improvement in painful diabetic neuropathy^[41]. Oxaliplatin-induced peripheral neurotoxicity in the peripheral nerve shows several histological characteristics including demyelination and degeneration of nerve fibers, and decrease in the number of myelinated fibers^[42]. In this study, oxaliplatin caused sciatic nerves of the oxaliplatin-treated group showed axonal degeneration and decreased density of myelinated fibers. However, these histological changes were not ameliorated in the tissue of rats treated with co-administration of oxaliplatin and VPA. It suggested that VPA was unable to prevent demyelination and degeneration of nerve fibers from oxaliplatin-induced peripheral neurotoxicity.

Oxaliplatin causes damage to cell bodies and selective atrophy of subpopulations of DRG neurons^[43]. In previous evidence^[44], it is demonstrated that DRGs are a primary target for oxaliplatin neurotoxicity. In the present study, the histological determinations were performed on lumbar DRGs from oxaliplatin-treated rats, VPA exerted a significant protective effect by reducing the occurrence of multinucleolated neurons and the nucleolar eccentricity caused by oxaliplatin. VPA prevented morphological derangements in DRGs from oxaliplatin-treated rats, showed the same effects of N-Palmitoylethanolamine in oxaliplatin-treated rats^[3].

Besides the neuronal damage, glial cells have recently been recognized as a powerful modulator of pain. The activation of spinal astrocytes has been reported to be involved in the oxaliplatin-induced neuropathic pain^[45,46]. In models of trauma-induced neuropathy, microglia appear to exert a key role in the initial phases of neuropathic pain whereas astrocytes may be involved in its maintenance^[47,48]. In addition, glial inhibitors have been described as pain relievers and glial cells are emerging as a new target for drug development^[49]. The increased cell density of astrocyte and microglia is strongly related to pain hypersensitivity since the glial inhibitor minocycline and fluorocitrate fully prevent oxaliplatin-evoked pain^[38].

N-Palmitoylethanolamine has been reported to modulate glial cells and exert antinociceptive effects on oxaliplatin-induced neuropathic pain in rats^[3]. Furthermore, Kimura *et al*^[50,51] reported VPA (300 mg/

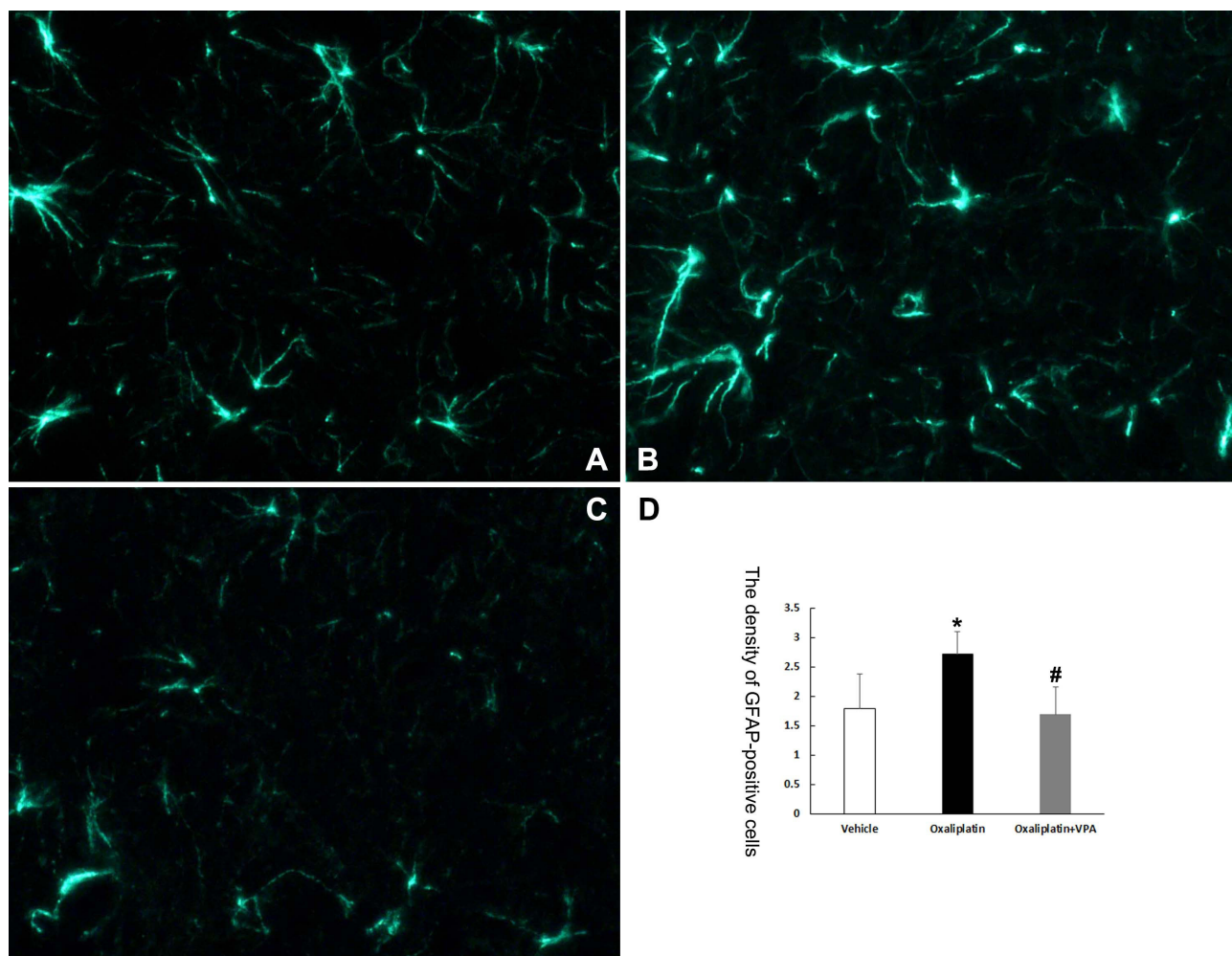


Figure 6 Astrocytes activation profile in the lumbar 4-5 spinal cord. The effect of repeated treatment with VPA was evaluated in oxaliplatin-treated rats on day 28. The density of GFAP-positive cells was calculated by means of the automatic thresholding and segmentation features of ImageJ (ImageJ 1.50a; Wayne Rasband, National Institutes of Health, MD, USA) in (D). Images (original magnification 200 x) of sections showed vehicle in (A), oxaliplatin in (B) and oxaliplatin + VPA in (C). Each value represents the mean of 6 rats per group, performed in two different experimental sets. * $P < 0.05$ versus vehicle; # $P < 0.05$ versus oxaliplatin.

kg) was administered via intraperitoneal injection in a conditional knockout mice, which exhibit glaucomatous pathology including glutamate neurotoxicity and oxidative stress in the retina, daily for 2 weeks. It showed VPA prevents retinal degeneration in a murine model of normal tension glaucoma. Subcutaneous injections of 300 mg/kg VPA twice a day, VPA-mediated neuroprotection against I/R injury in the retina may involve cytoprotective Hsp70 induction via transcriptional activation and inhibition of the mitochondria-mediated apoptosis pathway^[40]. In developing brain, few studies have examined VPA effects on glial cells, particularly astrocytes^[36].

In the present study, to examine the effect of VPA on oxaliplatin-induced glial activation on spinal cord, immunohistochemical evaluation using GFAP and Iba1 antibodies was performed. On day 28, a lower pain threshold was accompanied by effects on spinal astrocytes and microglia that involve a significant increase of the number of cells immunoreactive to GFAP and Iba1, respectively. The present results reveal an inhibitory effect of VPA on astrocytes and microglia in the dorsal horn of the spinal cord with decreasing in the number or activation of both cell types. It suggested that VPA-ameliorated oxaliplatin-induced astrocytes and microglia activation, meaning that proinflammatory mediator-related nociceptor sensitization could be

prevented by VPA administration.

In conclusion, the present results demonstrate for the first time that VPA administration ameliorated the oxaliplatin-induced behavioral, biochemical and histopathological changes in rats. The VPA-mediated effects in this study may be attributed to neuroprotection properties and ameliorating oxaliplatin-induced astrocytes and microglial activation. VPA may offer a dual protective approach against etiological factors and resulting maladaptive plasticity. However, further study is needed to evaluate the effect of VPA on several symptoms of oxaliplatin-induced peripheral neuropathy and the mechanism of VPA on biomolecular changes of oxaliplatin-induced neuropathy. To get more conclusive results, a larger number of rats in each group and more elaborate techniques are needed.

ACKNOWLEDGMENTS

The authors wish to acknowledge Support Center for Medical Research and Education, Tokai University, for technical support of Department of Cell Biology and Histology analysis with Nahoko Fukunishi and Masayoshi Tokunaga. Part of this study was supported by a Grant-in-Aid for Scientific Research from the Ministry

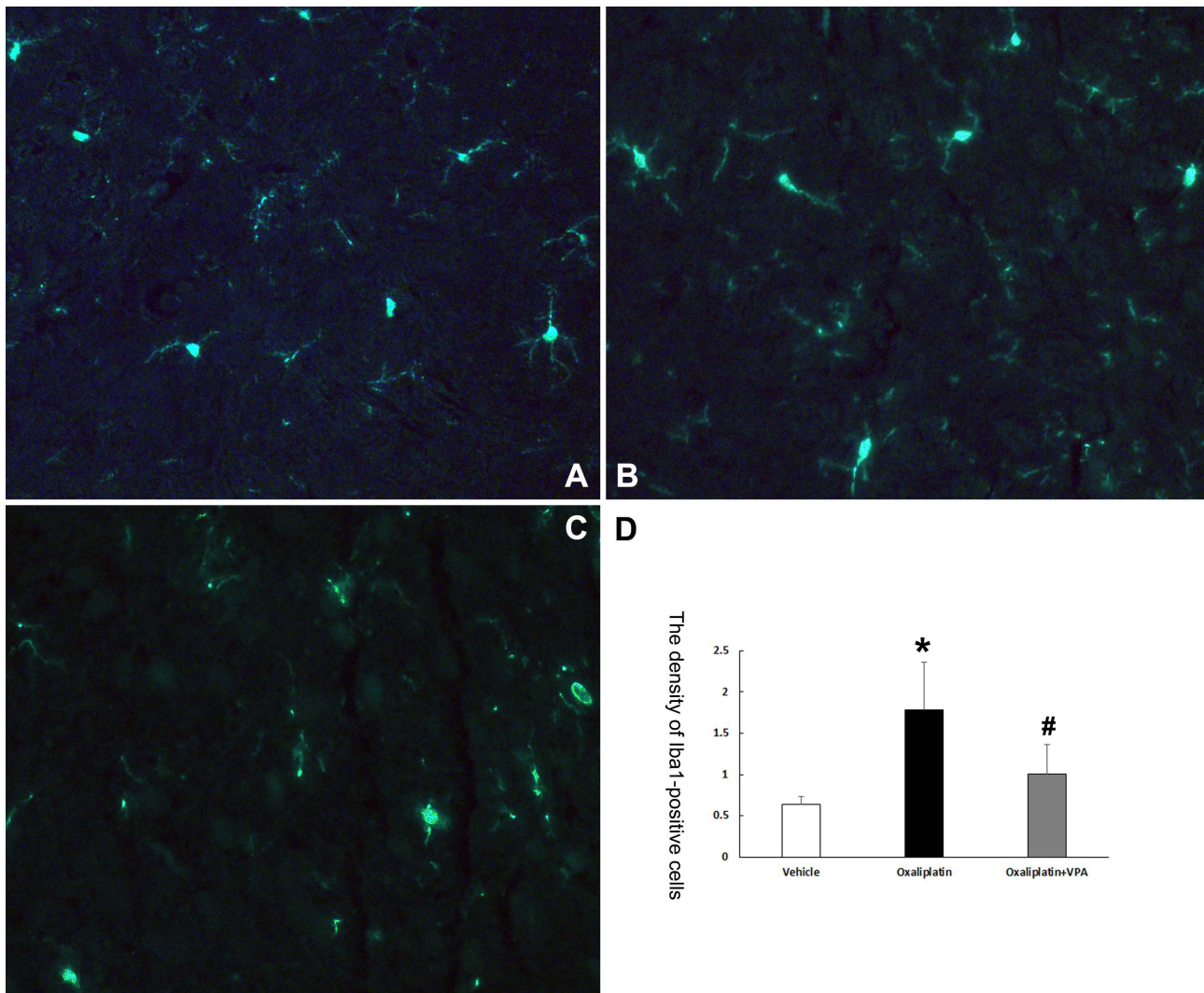


Figure 7 Microglia activation profile in the lumbar 4-5 spinal cord. The effect of repeated treatment with VPA was evaluated in oxaliplatin-treated rats on day 28. The density of Iba1-positive cells was calculated by means of the automatic thresholding and segmentation features of ImageJ (ImageJ 1.50a; Wayne Rasband, National Institutes of Health, MD, USA) in (D). Images (original magnification 200 x) of sections showed vehicle in (A), oxaliplatin in (B) and oxaliplatin + VPA in (C). Each value represents the mean of 6 rats per group, performed in two different experimental sets. * $P < 0.05$ versus vehicle; # $P < 0.05$ versus oxaliplatin.

of Education, Culture, Sports, Science and Technology of Japan (Nos. 22590171 and 25460265), and a Grant for Scientific Research from Faculty of Health Sciences, Tokyo Metropolitan University (No.280530-1425000).

REFERENCES

- Okazaki S, Schirripa M, Loupakis F, Cao S, Zhang W, Yang D, Ning Y, Berger MD, Miyamoto Y, Suenaga M, Iqbal S, Barzi A, Cremlini C, Falcone A, Battaglin F, Salvatore L, Borelli B, Helentjaris TG, Lenz HJ. Tandem repeat variation near the HIC1 (hypermethylated in cancer 1) promoter predicts outcome of oxaliplatin-based chemotherapy in patients with metastatic colorectal cancer. *Cancer* 2017; Nov 15; **123**(22): 4506-4514. [PMID: 28708932]; [DOI: 10.1002/ncr.30880]
- Kono T, Suzuki Y, Mizuno K, Miyagi C, Omiya Y, Sekine H, Mizuhara Y, Miyano K, Kase Y, Uezono Y. Preventive effect of oral goshajinkigan on chronic oxaliplatin-induced hypoesthesia in rats. *Sci Rep* 2015; **5**: 16078. [PMID: 26542342]; [DOI: 10.1038/srep16078]
- Di Cesare Mannelli L, Pacini A, Corti F, Boccella S, Luongo L, Esposito E, Cuzzocrea S, Maione S, Calignano A, Ghelardini C. Antineuropathic profile of N-palmitoylethanolamine in a rat model of oxaliplatin-induced neurotoxicity. *PLoS One* 2015; **10**: e0128080. [PMID: 26039098]; [DOI: 10.1371/journal.pone.0128080]
- Grothey A. Clinical management of oxaliplatin-associated neurotoxicity. *Clin Colorectal Cancer* 2005; **5 Suppl 1**: S38-46. [PMID: 15871765]
- Pachman DR, Qin R, Seisler DK, Smith EM, Beutler AS, Ta LE, Lafky JM, Wagner-Johnston ND, Ruddy KJ, Dakhil S, Staff NP, Grothey A, Loprinzi CL. Clinical course of oxaliplatin-induced neuropathy: results from the randomized phase III trial N08CB (Alliance). *J Clin Oncol* 2015; **33**: 3416-3422. [PMID: 26282635]; [DOI: 10.1200/JCO.2014.58.8533]
- Kawashiri T, Egashira N, Watanabe H, Ikegami Y, Hirakawa S, Mihara Y, Yano T, Ikesue H, Oishi R. Prevention of oxaliplatin-induced mechanical allodynia and neurodegeneration by neurotrophin in the rat model. *Eur J Pain* 2011; **15**: 344-350. [PMID: 20829082]; [DOI: 10.1016/j.ejpain.2010.08.006]
- Broomand A, Jerremalm E, Yachnin J, Ehrsson H, Elinder F. Oxaliplatin neurotoxicity--no general ion channel surface-charge effect. *J Negat Results Biomed* 2009; **8**: [DOI: 10.1186/1477-5751-8-2]
- Fujita S, Ushio S, Ozawa N, Masuguchi K, Kawashiri T, Oishi R, Egashira N. Exenatide facilitates recovery from oxaliplatin-induced peripheral neuropathy in rats. *PLoS One* 2015; **10**: e0141921. [PMID: 26536615]; [DOI: 10.1371/journal.pone.0141921]
- Beijers AJ, Mols F, Vreugdenhil G. A systematic review on chronic oxaliplatin-induced peripheral neuropathy and the relation with oxaliplatin administration. *Support Care Cancer* 2014; **22**: 1999-2007. [PMID: 24728618]; [DOI: 10.1007/s00520-014-2242-z]
- Ohsawa M, Otake S, Murakami T, Yamamoto S, Makino T, Ono H. Gabapentin prevents oxaliplatin-induced mechanical hyperalgesia in mice. *J Pharmacol Sci* 2014; **125**: 292-299. [PMID: 24990115]
- von Delius S, Eckel F, Wagenpfeil S, Mayr M, Stock K, Kullmann F, Obermeier F, Erdmann J, Schmelz R, Quasthoff S, Adelsberger H, Breidenkamp R, Schmid RM, Lersch C. Carbamazepine for prevention of oxaliplatin-related neurotoxicity in patients with advanced colorectal cancer: final results of a randomised, controlled, multicenter phase II study. *Invest New Drugs* 2007; **25**: 173-180. [PMID: 16983507]; [DOI: 10.1007/s10637-006-9010-y]
- Kim ST, Chung YH, Lee HS, Chung SJ, Lee JH, Sohn UD, Shin YK, Park ES, Kim HC, Bang JS, Jeong JH. Protective effects of phosphatidylcholine on oxaliplatin-induced neuropathy in rats. *Life Sci* 2015; **130**: 81-87. [PMID: 25817232]; [DOI: 10.1016/j.lfs.2015.03.013]
- Cascinu S, Catalano V, Cordella L, Labianca R, Giordani P, Baldelli AM, Beretta GD, Ubiali E, Catalano G. Neuroprotective effect of reduced glutathione on oxaliplatin-based chemotherapy in advanced colorectal cancer: a randomized, double-blind, placebo-controlled trial. *J Clin Oncol* 2002; **20**: 3478-3483. [PMID: 12177109]; [DOI: 10.1200/JCO.2002.07.061]
- Smith EM, Pang H, Cirrincione C, Fleishman S, Paskett ED, Ahles T, Bressler LR, Fadul CE, Knox C, Le-Lindqwister N, Gilman PB, Shapiro CL; Alliance for Clinical Trials in Oncology. Effect of duloxetine on pain, function, and quality of life among patients with chemotherapy-induced painful peripheral neuropathy: a randomized clinical trial. *JAMA* 2013; **309**: 1359-1367. [PMID: 23549581]; [DOI: 10.1001/jama.2013.2813]
- Afonseca SO, Cruz FM, Cubero Dde I, Lera AT, Schindler F, Okawara M, Souza LF, Rodrigues NP, Giglio Ad. Vitamin E for prevention of oxaliplatin-induced peripheral neuropathy: a pilot randomized clinical trial. *Sao Paulo Med J* 2013; **131**: 35-38. [PMID: 23538593]
- Gaskell H, Moore RA, Derry S, Stannard C. Oxycodone for neuropathic pain and fibromyalgia in adults. *Cochrane Database Syst Rev* 2014; **6**: CD010692. [PMID: 24956205]; [DOI: 10.1002/14651858.CD010692.pub2]
- Oki E, Emi Y, Kojima H, Higashijima J, Kato T, Miyake Y, Kon M, Ogata Y, Takahashi K, Ishida H, Saeki H, Sakaguchi Y, Yamanaka T, Kono T, Tomita N, Baba H, Shirabe K, Kakeji Y, Maehara Y. Preventive effect of Goshajinkigan on peripheral neurotoxicity of FOLFOX therapy (GENIUS trial): a placebo-controlled, double-blind, randomized phase III study. *Int J Clin Oncol* 2015; **20**: 767-775. [PMID: 25627820]; [DOI: 10.1007/s10147-015-0784-9]
- de Andrade DC, Jacobsen Teixeira M, Galhardoni R, Ferreira KSL, Braz Mileno P, Scisci N, Zandonai A, Teixeira WGJ, Saragiotto DF, Silva V, Raicher I, Cury RG, Macareno R, Otto Heise C, Wilson Iervolino Brotto M, Andrade de Mello A, Zini Megale M, Henrique Curti Dourado L, Mendes Bahia L, Lilian Rodrigues A, Parravano D, Tizue Fukushima J, Lefaucheur JP, Bouhassira D, Sobroza E, Riechelmann RP, Hoff PM; PreOx Workgroup, Valério da Silva F, Chile T, Dale CS, Nebuloni D, Senna L, Brentani H, Pagano RL, de Souza ÂM. Pregabalin for the prevention of oxaliplatin-induced painful neuropathy: a randomized, double-blind trial. *Oncologist* 2017 Oct; **22**(10): 1154-e105. pii: [PMID: 28652279]; [DOI: 10.1634/theoncologist.2017-0235]
- Bruna J, Videla S, Argyriou AA, Velasco R, Villoria J, Santos C, Nadal C, Cavaletti G, Alberti P, Briani C, Kalofonos HP, Cortinovis D, Sust M, Vaqué A, Klein T, Plata-Salamán C. Efficacy of a novel sigma-1 receptor antagonist for oxaliplatin-induced neuropathy: A randomized, double-blind, placebo-controlled phase IIa clinical trial. *Neurotherapeutics* 2017; [PMID: 28924870]; [DOI: 10.1007/s13311-017-0572-5]
- Rakitin A, Köks S, Haldre S. Metabolic syndrome and anticonvulsants: A comparative study of valproic acid and carbamazepine. *Seizure* 2016; **38**: 11-16. [PMID: 27061880]; [DOI: 10.1016/j.seizure.2016.03.008]
- Chen L, Cui X, Wu Z, Jia L, Yu Y, Zhou Q, Hu X, Xu W, Luo D, Liu J, Xiao J, Yan Q, Cheng L. Transplantation of bone marrow mesenchymal stem cells pretreated with valproic acid in rats with an acute spinal cord injury. *Biosci Trends* 2014; **8**: 111-119. [PMID: 24815388]
- Abdanipour A, Schluesener HJ, Tiraihi T, Noori-Zadeh A. Systemic administration of valproic acid stimulates overexpression of microtubule-associated protein 2 in the spinal cord injury model to promote neurite outgrowth. *Neurol Res* 2015; **37**: 223-228. [PMID: 25203772]; [DOI: 10.1179/1743132814Y.0000000438]
- Hamming AM, van der Toorn A, Rudrapatna US, Ma L, van Os

- HJ, Ferrari MD, van den Maagdenberg AM, van Zwet E, Poinatte K, Stowe AM, Dijkhuizen RM, Wermer MJ. Valproate reduces delayed brain injury in a rat model of subarachnoid hemorrhage. *Stroke* 2017; **48**: 452-458. [PMID: 28028144]; [DOI: 10.1161/STROKEAHA.116.014738]
24. Bambakidis T, Dekker SE, Sillesen M, Liu B, Johnson CN, Jin G, de Vries HE, Li Y, Alam HB. Resuscitation with valproic acid alters inflammatory genes in a porcine model of combined traumatic brain injury and hemorrhagic shock. *J Neurotrauma* 2016; **33**: 1514-1521. [PMID: 26905959]; [DOI: 10.1089/neu.2015.4163]
 25. Lee JY, Maeng S, Kang SR, Choi HY, Oh TH, Ju BG, Yune TY. Valproic acid protects motor neuron death by inhibiting oxidative stress and endoplasmic reticulum stress-mediated cytochrome C release after spinal cord injury. *J Neurotrauma* 2014; **31**: 582-594. [PMID: 24294888]; [DOI: 10.1089/neu.2013.3146]
 26. Carriere CH, Kang NH, Niles LP. Neuroprotection by valproic acid in an intrastriatal rotenone model of Parkinson's disease. *Neuroscience* 2014; **267**: 114-121. [PMID: 24613722]; [DOI: 10.1016/j.neuroscience.2014.02.028]
 27. Yao ZG, Jing HY, Wang DM, Lv BB, Li JM, Liu FF, Fan H, Sun XC, Qin YJ, Zhao MQ. Valproic acid ameliorates olfactory dysfunction in APP/PS1 transgenic mice of Alzheimer's disease: Ameliorations from the olfactory epithelium to the olfactory bulb. *Pharmacol Biochem Behav* 2016; **144**: 53-59. [PMID: 26948859]; [DOI: 10.1016/j.pbb.2016.02.012]
 28. Chiu CT, Liu G, Leeds P, Chuang DM. Combined treatment with the mood stabilizers lithium and valproate produces multiple beneficial effects in transgenic mouse models of Huntington's disease. *Neuropsychopharmacology* 2011; **36**: 2406-2421. [PMID: 21796107]; [DOI: 10.1038/npp.2011.128]
 29. Hoffmann K, Czapp M, Löscher W. Increase in antiepileptic efficacy during prolonged treatment with valproic acid: role of inhibition of histone deacetylases? *Epilepsy Res* 2008; **81**: 107-113. [PMID: 18538545]; [DOI: 10.1016/j.eplepsyres.2008.04.019]
 30. van Bergeijk J, Haastert K, Grothe C, Claus P. Valproic acid promotes neurite outgrowth in PC12 cells independent from regulation of the survival of motoneuron protein. *Chem Bio Drug Des* 2006; **67**: 244-247. [PMID: 16611218]; [DOI: 10.1111/j.1747-0285.2006.00369.x]
 31. Cournoyer P, Desrosiers RR. Valproic acid enhances protein L-isoaspartyl methyltransferase expression by stimulating extracellular signal-regulated kinase signaling pathway. *Neuropharmacology* 2009; **56**: 839-848. [PMID: 19371592]; [DOI: 10.1016/j.neuropharm.2009.01.008]
 32. Masuch A, Shieh CH, van Rooijen N, van Calker D, Biber K. Mechanism of microglia neuroprotection: Involvement of P2X7, TNF α , and valproic acid. *Glia* 2016; **64**: 76-89. [PMID: 26295445]; [DOI: 10.1002/glia.22904]
 33. International Association for the Study of Pain curricula (IASP) [<https://www.iasp-pain.org/Education/Content.aspx?ItemNumber=1213>],
 34. Ling B, Authier N, Balayssac D, Eschalier A, Coudore F. Behavioral and pharmacological description of oxaliplatin-induced painful neuropathy in rat. *Pain* 2007; **128**: 225-234. [PMID: 17084975]; [DOI: 10.1016/j.pain.2006.09.016]
 35. Uchida H, Matsushita Y, Araki K, Mukae T, Ueda H. Histone deacetylase inhibitors relieve morphine resistance in neuropathic pain after peripheral nerve injury. *J Pharmacol Sci* 2015; **128**: 208-211. [PMID: 26318673]; [DOI: 10.1016/j.jphs.2015.07.040]
 36. Lee HJ, Dreyfus C, DiCicco-Bloom E. Valproic acid stimulates proliferation of glial precursors during cortical gliogenesis in developing rat. *Dev Neurobiol* 2016; **76**: 780-798. [PMID: 26505176]; [DOI: 10.1002/dneu.22359]
 37. Ushio S, Egashira N, Sada H, Kawashiri T, Shirahama M, Masuguchi K, Oishi R. Goshajinkigan reduces oxaliplatin-induced peripheral neuropathy without affecting anti-tumour efficacy in rodents. *Eur J Cancer* 2012; **48**: 1407-1413. [PMID: 21907570]; [DOI: 10.1016/j.ejca.2011.08.009]
 38. Di Cesare Mannelli L, Pacini A, Micheli L, Tani A, Zanardelli M, Ghelardini C. Glial role in oxaliplatin-induced neuropathic pain. *Exp Neurol* 2014; **261**: 22-33. [PMID: 24967684]; [DOI: 10.1016/j.expneurol.2014.06.016]
 39. Yi SQ, Li J, Yamaguchi T, Hori K, Hayashi K, Itoh M, Ozaki N. Immunolocalization of the PP family and its receptors in the gastrointestinal tract of house musk shrew, *Suncus murinus*. *Neuro Endocrinol Lett* 2011; **312**: 212-219. [PMID: 21552195]
 40. Zhang Z, Qin X, Tong N, Zhao X, Gong Y, Shi Y, Wu X. Valproic acid-mediated neuroprotection in retinal ischemia injury via histone deacetylase inhibition and transcriptional activation. *Exp Eye Res* 2012; **94**: 98-108. [PMID: 22143029]; [DOI: 10.1016/j.exer.2011.11.013]
 41. Kochar DK, Rawat N, Agrawal RP, Vyas A, Beniwal R, Kochar SK, Garg P. Sodium valproate for painful diabetic neuropathy: a randomized double-blind placebo-controlled study. *QJM* 2004; **97**: 33-38. [PMID: 14702509]
 42. Di Cesare Mannelli L, Zanardelli M, Failli P, Ghelardini C. Oxaliplatin-induced neuropathy: oxidative stress as pathological mechanism. Protective effect of silibinin. *J Pain* 2012; **13**: 276-284. [PMID: 22325298]; [DOI: 10.1016/j.jpain.2011.11.009]
 43. Jamieson SM, Liu J, Connor B, McKeage MJ. Oxaliplatin causes selective atrophy of a subpopulation of dorsal root ganglion neurons without inducing cell loss. *Cancer Chemother Pharmacol* 2005; **56**: 391-399. [PMID: 15887017]; [DOI: 10.1007/s00280-004-0953-4]
 44. Di Cesare Mannelli L, Bonaccini L, Mello T, Zanardelli M, Pacini A, Ghelardini C. Morphologic features and glial activation in rat oxaliplatin-dependent neuropathic pain. *J Pain* 2013; **14**: 1585-1600. [PMID: 24135431]; [DOI: 10.1016/j.jpain.2013.08.002]
 45. Yoon SY, Robinson CR, Zhang H, Dougherty PM. Spinal astrocyte gap junctions contribute to oxaliplatin-induced mechanical hypersensitivity. *J Pain* 2013; **14**: 205-214. [PMID: 23374942]; [DOI: 10.1016/j.jpain.2012.11.002]
 46. Di Cesare Mannelli L, Pacini A, Matera C, Zanardelli M, Mello T, De Amici M, Dallanocce C, Ghelardini C. Involvement of $\alpha 7$ nAChR subtype in rat oxaliplatin-induced neuropathy: effects of selective activation. *Neuropharmacology* 2014; **79**: 37-48. [PMID: 24225197]; [DOI: 10.1016/j.neuropharm.2013.10.034]
 47. Marchand F, Perretti M, McMahon SB. Role of the immune system in chronic pain. *Nat Rev Neurosci* 2005; **6**: 521-532. [PMID: 15995723]; [DOI: 10.1038/nrn1700]
 48. Scholz J, Woolf CJ. The neuropathic pain triad: neurons, immune cells and glia. *Nat Neurosci* 2007; **10**: 1361-1368. [PMID: 17965656]; [DOI: 10.1038/nn1992]
 49. Sung CS, Cherng CH, Wen ZH, Chang WK, Huang SY, Lin SL, Chan KH, Wong CS. Minocycline and fluorocitrate suppress spinal nociceptive signaling in intrathecal IL-1 β -induced thermal hyperalgesic rats. *Glia* 2012; **60**: 2004-2017. [PMID: 22972308]; [DOI: 10.1002/glia.22415]
 50. Kimura A, Guo X, Noro T, Harada C, Tanaka K, Namekata K, Harada T. Valproic acid prevents retinal degeneration in a murine model of normal tension glaucoma. *Neurosci Lett* 2015; **588**: 108-113. [PMID: 25555796]; [DOI: 10.1016/j.neulet.2014.12.054]
 51. Kimura A, Namekata K, Guo X, Noro T, Harada C, Harada T. Valproic acid prevents NMDA-induced retinal ganglion cell death via stimulation of neuronal TrkB receptor signaling. *Am J Pathol* 2015; **185**: 756-764. [PMID: 25542970]; [DOI: 10.1016/j.ajpath.2014.11.005]