INVITED REVIEW

Goshajinkigan attenuates paclitaxel-induced neuropathic pain via cortical astrocytes

Kenta Takanashi1 | Keisuke Shibata1 | Keita Mizuno1,2 | Ryohei Komatsu1 | Schuichi Koizumi1,3

1Department of Neuropharmacology, Interdisciplinary Graduate School of Medicine, University of Yamanashi, Chuo, Yamanashi, Japan
2Tsumura Research Laboratories, Kampo Scientific Strategies Division, Tsumura & Co, Inashiki-gun, Japan
3Yamanashi GLIA Center, University of Yamanashi, Yamanashi, Japan

Correspondence
Schuichi Koizumi, Department of Neuropharmacology, Interdisciplinary Graduate School of Medicine, University of Yamanashi, 1110 Shimokato, Chuo, Yamanashi 409-3898, Japan.
Email: skoizumi@yamanashi.ac.jp

Funding information
This work was supported by JSPS Grants-in-aid for Scientific Research (KAKENHI) (JP25117003, 18H0512, 19H04746, 20H05060, 20H05902, 21H04786, and 21K19309 to S.K.), AMED-CREST (JP20gm1310008 to SK), Takeda Science Foundation (to SK), The Mitsubishi Foundation (to SK), and Frontier in Brain Science Program in Univ Yamanashi (to SK).

Abstract
The anticancer agents platinum derivatives and taxanes such as paclitaxel (PCX) often cause neuropathy known as chemotherapy-induced peripheral neuropathy with high frequency. However, the cellular and molecular mechanisms underlying such neuropathy largely remain unknown. Here, we show new findings that the effect of Goshajinkigan (GJG), a Japanese KAMPO medicine, inhibits PCX-induced neuropathy by acting on astrocytes. The administration of PCX in mice caused the sustained neuropathy lasting at least 4 weeks, which included mechanical allodynia and thermal hyperalgesia but not cold allodynia. PCX-evoked pain behaviors were associated with the sensitization of all primary afferent fibers. PCX did not activate microglia or astrocytes in the spinal cord. However, it significantly activated astrocytes in the primary sensory (S1) cortex without affecting S1 microglial activation there. GJG significantly inhibited the PCX-induced mechanical allodynia by 50% and thermal hyperalgesia by 90%, which was in accordance with the abolishment of astrocytic activation in the S1 cortex. Finally, the inhibition of S1 astrocytes by an astrocyte-toxin L-alpha-aminoadipic acid abolished the PCX-induced neuropathy. Our findings suggest that astrocytes in the S1 cortex would play an important role in the pathogenesis of PCX-induced neuropathy and are a potential target for its treatment.

KEYWORDS
astrocytes, chemotherapy-induced neuropathy, cortex, KAMPO medicine, primary somatosensory cortex

1 | INTRODUCTION

Chemotherapy is the mainstay of cancer treatment. However, platinum-based anticancer drugs and taxanes such as paclitaxel (PCX) frequently cause chemotherapy-induced peripheral neuropathy (CIPN), which involves numbness, abnormal sensation, sensory dullness, and pain as side effects.1 CIPN is a dose-limiting factor in cancer chemotherapy, and when it becomes severe, it prevents a

Abbreviations: BBB, blood–brain barrier; CIPN, chemotherapy-induced peripheral neuropathy; GJG, Goshajinkigan; LAAA, L-alpha-aminoadipic acid; PBS, phosphate-buffered saline; PCX, paclitaxel; PFA, paraformaldehyde.

Kenta Takanashi and Keisuke Shibata have equal contribution.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. Pharmacology Research & Perspectives published by British Pharmacological Society and American Society for Pharmacology and Experimental Therapeutics and John Wiley & Sons Ltd.
major obstacle to continued cancer treatment because patients are forced to reduce or withdraw anticancer drugs. Furthermore, CIPN often persists or becomes refractory, even after the withdrawal of anticancer drugs. CIPN is treated with pregabalin, non-steroidal anti-inflammatory drugs, and opioids; however, the efficacy of these drugs is inadequate. There are currently no effective prophylactic or therapeutic agents for the treatment of CIPN, and this is an area of extremely high unmet need. Histological studies have demonstrated that the pathogenesis of CIPN is attributed to direct neuronal axonal or cell body injury. However, the underlying mechanisms have not yet been elucidated. Furthermore, it is difficult to determine the molecular pathogenesis of CIPN by conducting conventional experiments that are primarily focused on neurons, especially for the development of therapies for remission of symptoms that have already developed and become intractable. Recent research on intractable pain conditions, such as neuropathic pain, has strongly suggested the importance of spinal glia and cortical glial cells.

Japanese KAMPO medicines, which are traditional herbal medicines, have received substantial attention for their effectiveness in addressing the abovementioned unmet needs and are approved for clinical use by the National Health Insurance Program in Japan. Goshajinkigan (GJG) is a form of KAMPO medicine that is composed of a fixed formulation of 10 herbal drugs; Rehmanniae Radix, Achyranthis Radix, Corni Fructus, Dioscoreae Rhizoma, Plantaginis Semen, Alismatis Rhizoma, Poria, Moutan Cortex, Cinnamomum Cortex, and Processi Aconiti Radix. Although GJG is used widely for the treatment of various diseases, it is commonly used for the treatment of lower back pain, numbness, and neuropathy, which includes CIPN. Several clinical studies have shown that GJG is effective for neuropathy induced by chemotherapies. Notably, GJG suppresses CIPN without impairing the antitumor activity of anticancer drugs. However, the mechanism of CIPN suppression by GJG remains poorly understood. Previously, we showed that Bushi (Processi Aconiti Radix), one of the herbal drugs contained in GJG, has a very strong inhibitory effect on neuropathic pain induced by peripheral nerve injury. Moreover, the analgesic effect of Bushi is observed even after it is administered in the chronic phase of pain or following the withdrawal of chemicals. Importantly, the effect is induced by the inhibition of glial (astrocyte) activation in the spinal cord. The glial perspective is important in understanding CIPN. Therefore, we aimed to elucidate the analgesic effect of GJG on PCX-induced neuropathy in mice from the glial perspective. Although used widely in the treatment of solid tumor, PCX causes painful neuropathy in 97% of patients, which becomes chronic in 60% of patients. However, the detailed mechanisms underlying PCX-induced chronic neuropathy remain largely unknown.

In the present study, we demonstrated that in mice, PCX activates astrocytes in the somatosensory cortex but not in the spinal cord, and causes neuropathic pain, which includes hyperalgesia and mechanical allodynia. We also demonstrated that GJG inhibits both PCX-induced activation of astrocytes in the cortex and neuropathic pain, which suggests that cortical astrocytes are critical for understanding the molecular pathogenesis of CIPN and as a therapeutic target.

2 MATERIALS AND METHODS

2.1 Experimental schedule

PCX (Sigma-Aldrich) was dissolved in saline containing 10% Cremophor EL (Sigma-Aldrich) and 10% ethanol, which was administered intraperitoneally at a dosage of 4 mg/kg daily for 5 days. GJG used in the present study was supplied by Tsumura & CO. Plant materials were authenticated by identification of external morphology and marker compounds for plant specimens according to the methods of the Japanese Pharmacopeia and company standards. Extract quality was standardized based on good manufacturing practice as defined by the Ministry of Health, Labor and Welfare of Japan. GJG was dissolved in water, and was administered orally daily (from Day[0] to D28). Pain behaviors tests were performed 24 h after the final administration of GJG.

2.2 Assessment of cold allodynia

Cold allodynia induced by PCX was assessed using the acetone test. Approximately 50 µl of acetone (Wako Pure Chemical Ltd.) was sprayed onto the plantar skin of the right hind paw, and the time mice spent in the elevating and licking of the stimulated hind paw was measured for 60 s.

2.3 Assessment of thermal hyperalgesia

Thermal hyperalgesia was assessed by the plantar test as reported previously. In brief, the hind paw of the mice was stimulated with a radiant thermal stimulus apparatus (model 33, Analgesia Meter; IITC/Life Science Instruments). The latency of the paw withdrawal after the thermal stimulus was determined as the average of four measurements per paw. The intensity of the thermal stimulus was adjusted to achieve an average baseline paw withdrawal latency of about 9–11 s in control mice.

2.4 Assessment of mechanical allodynia

The von Frey test, which was used to assess mechanical allodynia, was performed according to the method described previously. Briefly, a 0.16 g of von Frey filament was applied to the plantar surface of the right hind paw. The paw withdrawal in response to the tactile stimulus was scored as follows: 0, no response; 1, a withdrawal response away from the stimulus with slight flinching and/or licking; and 2, an intense withdrawal response away from the stimulus with brisk flinching and/or licking. One trial comprised 10 applications of the filament, and each application was scored as a 0, 1, or 2. The trial was evaluated according to a total score of 0–20 at the end of the test (% of maximum score).
2.5 Measurement of current perception threshold

Individual stimulation of the three different primary afferent neurons (i.e., Aβ-, Aδ-, and C-fibers, was performed using the electrical stimulation-induced paw flexion method with a Neurometer (Neurotron Inc.) as reported previously. Briefly, electrodes (Neurotron Inc.) were attached to the right planter surfaces of the hind paw, and transcutaneous neuronal stimuli (sine-wave pulses of 5, 250, or 2000 Hz for activation of the C-, Aδ-, or Aβ-fibers, respectively) were applied. The minimum intensity (µA) at which each mouse showed a withdrawal response of the stimulated hind paw was defined as the current threshold.

2.6 Immunohistochemistry

After perfusion with saline and 4% paraformaldehyde (PFA), the lumbar spinal cord and brains were postfixed in 4% PFA for 24 h, and then permeated with 20% sucrose in 0.1 M phosphate-buffered saline (PBS) for 24 h and 30% sucrose in 0.1 M PBS for 48 h at 4°C. The lumbar spinal cord segments and brains were frozen in an embedding compound (Sakura Finetek) on dry ice. Frozen spinal segments were cut with a cryostat (Leica CM 1100; Leica) at a thickness of 30 µm and placed in PBS at 4°C to be processed immunohistochemically as free-floating sections. The sections were incubated overnight at 4°C with primary antibodies: mouse anti-glia fibrillary acidic protein (GFAP; 1:2000; Millipore) and rabbit anti-ionized calcium-binding adapter protein-1 (Iba1; 1:2000; Fujifilm-Wako). The sections were washed six times with 0.01 M PBS (10 min each) and then incubated for 3 h at room temperature with the secondary antibody: Alexa Fluor 488- and Alexa Fluor 546-conjugated mouse- and rabbit-IgGs (Invitrogen). Immunohistochemical images were obtained using a confocal laser microscope (Fluoview1000; Olympus) and digital images were captured with Fluoview1000 (Olympus).

2.7 Statistical analysis

All results are expressed as the means ± standard error of the mean. All statistical analyses were performed using the StatLight2000 software (Yukms Co., Ltd). All behavioral data, except for those of the von Frey test, were analyzed using a one-way analysis of variance (ANOVA) with post hoc multiple comparison using Tukey’s tests. Data for the von Frey test were analyzed using an ANOVA using Scheffé’s multiple comparison tests. Results with p values of <.05 were considered to be statistically significant.

3 RESULTS

3.1 PCX-induced neuropathy assessed by different indicators

First, PCX-evoked painful neuropathy was evaluated using several pain behavior tests. As shown in Figure 1A, PCX was administered to mice for 5 consecutive days, and then pain behavior tests were performed every week for 4 weeks. It has been reported that PCX causes various pain behaviors. Therefore, we focused on mechanical allodynia, thermal hyperalgesia, and cold allodynia, which were assessed using von Frey test (Figure 1B), planter test (Figure 1C), and acetone test, respectively. On day 7 (D7), PCX significantly gradually increased both mechanical allodynia and thermal hypersensitivity, which lasted at least for 4 weeks (until at D28). PCX did not cause cold allodynia according to the acetone test (Figure 1D). Chronic treatment with GJG, significantly reduced PCX-evoked pain both behaviors after D21 (mechanical allodynia) and D14 (thermal hyperalgesia).

To determine which types of primary afferent neurons are involved in the PCX-evoked painful neuropathy, we assessed the changes in sensitivity of three different sensory fibers, which reflects the effect of GJG. Similar to the report of Matsumoto et al., we differentially stimulated the Aβ-, Aδ-, and C-fibers at frequencies of 2000, 250, and 5 Hz, respectively. We measured the minimum intensity of each stimulation at which the animal withdrew their hind limb. PCX significantly decreased the paw flexion threshold, which was mediated by all sensory fibers (Figure 1E-G), and was restored by the treatment with GJG.

We previously showed that in the spinal cord, both Iba1-positive microglia and GFAP-positive astrocytes are activated by several chronic pain models such as sciatic nerve ligation (SNL), which changes the quality of the synaptic transmission of sensory neurons. Such activation of spinal glial cells is one of the causes of neuropathic pain. Indeed, when spinal astrocytes were inhibited by Bushi, one of the active ingredients in GJG, the SNL-evoked mechanical allodynia was dramatically inhibited. However, PCX did not activate spinal astrocytes (Figure 2A B) during the observation period (from D7 to D28) or spinal microglia (Figure 2A C). The administration of GJG did not affect GFAP- or Iba1-positive signals in the spinal cord (Figure 2).

Recently, we found that SNL-activated astrocytes in the primary sensory (S1) cortex, which subsequently induced mechanical allodynia by increasing uncontrolled synaptogenesis, and this led to the mis connection between the tactile and pain networks of the S1 cortex. Therefore, we tested whether PCX activates astrocytes in the S1 astrocytes using immunohistochemistry. Twenty-one days after PCX administration, GFAP-positive signals were significantly increased, whereas Iba1-positive signals were not (Figure 3A C). The administration of GJG significantly reduced the enhanced GFAP-positive signals without affecting the Iba1-positive signals (Figure 3A C). PCX-activated S1 astrocytes without affecting spinal astrocytes and microglia or S1 microglia, and GJG almost completely suppressed the activation of S1 astrocytes. Moreover, there appeared to be a close correlation between the activation of S1 astrocytes and PCX-induced neuropathic pain behaviors, and between GJG-induced inhibition of astrocytic activation in the S1 cortex and pain behaviors.

We then investigated the causality between the activation of S1 astrocytes and pain behaviors. L-alpha-aminoacidic acid (LAAA) is an astrocyte-specific gliotoxin, and treatment with LAAA abolished the PCX-induced activation of astrocytes in the S1 cortex without affecting the Iba1-positive signals (Figure 4A C). Furthermore, LAAA almost completely inhibited PCX-evoked mechanical allodynia, as assessed by von Frey test, almost completely, without affecting the behaviors
**FIGURE 1** Various pain behaviors induced by PCX, showing the effect of GJG. (A) Schedule of PCX-treatment, GJG administration, and behavior tests. PCX (4 mg/kg, i.p.) was injected daily for the initial consecutive 5 days (D1–D5). GJG was orally administered daily throughout the experiments. Pain behavior tests were performed 24 h after GJG administration on D7, D14, D21, and D28. (B–D) Time course of PCX-induced pain behaviors. Mechanical allodynia was assessed using the von Frey test (0.166 g: B); thermal hyperalgesia was assessed using the plantar test (C); and cold allodynia was assessed using the acetone test (D). (E–F) Time course of PCX-induced hyperactivation of sensory neurons: Aβ-fibers (2000 Hz; E), Aδ-fibers (250 Hz; F), and C-fibers (5 Hz; G) were assessed by a Neurometer. PCX sensitized all sensory fibers on all days tested (D7–D28 after initial PCX-injection). GJG significantly inhibited the sensitization of all sensory neurons. Data are expressed as means ± standard error of the mean (N = 6–17 per group as indicated). #p < .05, ##p < .01 compared with the vehicle-treated control group; $p < .05, $$p < .01$ compared with the PCX only-treated group.

**FIGURE 2** No glial activation induced by PCX in the spinal cord. (A) Representative immunohistochemical pictures of astrocytes (upper) and microglia (bottom) in the dorsal horn of the spinal cord stained with GFAP and Iba1 antibodies, respectively. Left, water-treated control group; middle, PCX-treated group; and right, PCX- and GJG-treated groups. (B–C) Summary of quantitative data of (A), showing the intensity of GFAP- (B) and Iba1- (C) positive signals. Data are expressed as means ± standard error of the mean (N = 3–7 per group as indicated).
of the vehicle-treated control mice (Figure 5). These findings strongly suggest that activation of S1 astrocytes is the cause of PCX-evoked mechanical allodynia, their suppression is directly related to treatment, and that GJG has the potential to act on them.

4 | DISCUSSION

In the present study, we demonstrated the following three points. First, PCX-induced neuropathy (i.e., mechanical allodynia and thermal hyperalgesia but not cold allodynia), which was associated with the activation of S1 astrocytes. Second, GJG inhibited the PCX-induced pain behaviors and activation of S1 astrocytes. Third, the inhibition of S1 astrocytes by an astrocyte toxin, LAAA abolished the PCX-induced pain behaviors. Taken together, these findings suggest that S1 astrocytes are involved in the PCX-induced neuropathy and are a potential target for treatment.

In the present study, we newly demonstrated that PCX activates S1 astrocytes. More importantly, we also demonstrated that such activation of S1 astrocytes is the cause of PCX-induced neuropathy because their inhibition led to the suppression of PCX-induced mechanical allodynia (Figure 5). We previously reported in a neuropathic pain model that SNL activates S1 astrocytes and that this activation is the cause of mechanical allodynia.3 This is consistent with the activation of S1 astrocytes in the present PCX-induced model and suggests that it may be a common pathology in some types of pain and neuropathy. However, despite the numerous lines of evidence showing the activation of spinal microglia or astrocytes by PCX, to the best of our knowledge, there have not been any reports on PCX- or other taxane-induced activation of S1 astrocytes. This may be because of differences in experimental conditions and species. However, the main reason is likely that pain research to date has not focused on brain glial cells. Previously, it was believed that PCX does not cross the blood–brain barrier (BBB) to access the brain parenchyma; it is now understood that PCX can cross the BBB and directly affect brain functions. Furthermore, the effect of PCX on the memory impairment17 has become a significant issue, and other psychiatric impairments induced by PCX, such as depression and anxiety, have also been reported.18,19 In the SNL model, the sciatic nerve was ligated away from the brain, which resulted in astrocytic activation in the S1 via the input neurons to that site. Thus, it is thought that the PCX-induced activation of S1 astrocytes may simply be due to the effects of PCX on the peripheral sensory neurons including Aβ-, Aδ-, and C-fibers (Figure 1), and their abnormalities cause astrocytic activation in the S1 input site. However, as mentioned above, it is also necessary to consider the central effects of PCX, such as the possibility that PCX reaches the brain and activates S1 astrocytes directly and that PCX entering the brain enhances the responsiveness of S1 astrocytes to peripheral input. Therefore, we hypothesize that the accumulation of PCX and abnormal input may act in an additive or synergistic manner to activate S1 astrocytes selectively. However, we must await further studies to clarify these. In any case, this study offers valuable insights as it demonstrates that PCX activates cortical astrocytes to induce neuropathy.
**FIGURE 4** Effect of LAAA on the PCX-evoked glial activation in the S1 cortex. (A) Representative immunohistochemical pictures of S1 astrocytes (upper) and microglia (bottom) stained with GFAP and Iba1 antibodies, respectively. Left, water-treated control group; Second from left, water plus LAAA-treated group; Second from right, PCX-treated group; Right, PCX- and GJG-treated groups. (B–C) Quantitative analysis of (A), showing the effect of LAAA on the intensity of GFAP- (B) and Iba1- (C) positive signals. Data are expressed as means ± standard error of the mean (N = 5–14 per group as indicated). **p < .01 compared with the vehicle-treated control group; $^{##}$p < .01 compared with the PCX only-treated group.

**FIGURE 5** Effect of LAAA on mechanical alldynia. The PCX-evoked mechanical alldynia was assessed using the von Frey test (0.166 g) to demonstrate the effect LAAA. Two weeks after the initial PCX injection, (4 mg/kg, i.p.), mice displayed mechanical alldynia behaviors. Subsequently, 1 day after LAAA treatment, at which point activation of S1 astrocytes was almost abolished, PCX-evoked mechanical alldynia was significantly inhibited. Data are expressed as means ± standard error of the mean (N = 5–14 per group, as indicated). **p < .01 compared with the vehicle-treated control group; $^{##}$p < .01 compared with the PCX only-treated group.
It was notable that PCX-activated S1 astrocytes without affecting microglial activation in both the S1 cortex and spinal cord. In the mouse SNL model, the surgery caused significant activation of both microglia and astrocytes in the spinal cord and S1, which resulted in mechanical allodynia and thermal hyperalgesia. In the spinal cord, the activation of microglia is strongly related to the neuropathic pain, but their contribution differs from that of astrocytes; microglia tend to contribute to the early acute phase of neuropathic pain, whereas astrocytes contribute to the late chronic phase of neuropathic pain. In the other pathological models, microglia are often activated initially, which is followed by astrocytic activation, and this is considered a universal phenomenon in the pathology. However, this is not always the case. In fact, in the S1 cortex, SNL surgery activates astrocytes but not microglia. In addition, here we did not observe PCX-evoked microglial activation in either the spinal cord or S1 cortex. The exact reason for the lack of microglial activation is unclear; however, microglial activation is not necessarily associated with chronic pain or CIPN. Rather, the activation of S1 astrocytes is significantly related to PCX-induced neuropathy. A previous report also showed that astrocytes, but not microglia, are activated by PCX, which contributes to the pathogenesis of PCX-evoked painful neuropathy. Thus, in some dosages or conditions, PCX may cause neuropathy without microglial activation.

As for the mechanisms underlying S1 astrocyte-mediated mechanical allodynia, we previously showed that in the SNL model that activation of S1 astrocytes produce synaptogenic molecules, such as thrombospondin-1, and excessive uncontrolled synapse formation, which causes the mismatch of the tactile and pain networks in the S1 networks. The results of the SNL model cannot be simply extrapolated to the results of our study because sciatic nerve injury differs from the systemic administration of PCX. However, because the activation of S1 astrocytes is a key outcome of both models, it is noteworthy that a molecular mechanism common to both models may be involved in CPX-induced neuropathy. Despite the numerous studies on the molecular mechanisms of CPX-induced neuropathy, the true etiology remains elusive. Many reports have suggested the involvement of inflammation. However, in the current model, microglia, of which their increase is a hallmark of central inflammation, were not activated. Therefore, careful analysis of the various molecular changes caused by the activation of S1 astrocytes is required to determine whether inflammation is indeed the etiology.

Numerous clinical and preclinical studies have been conducted on CIPN, and the mechanisms underlying the pathology have been demonstrated extensively. For example, taxanes such as PCX, have been shown to cause peripheral neuronal injuries by affecting microtubule dynamics, mitochondrial functions oxidative stress, and inflammation in the peripheral tissues, which leads to the injuries of peripheral sensory neurons. In regard to target molecules, a large variety of molecules and their dysfunctions are reported; ion channels, such as TRPV1, TRPA1, and TRPV4 channels; Ca2+ channels and Na+ channels; reactive oxygen species-related molecules, such as superoxide dismutase and glutathione-SH peroxidase and nuclear factor-2-erythroid-related factor-2; inflammatory molecules, such as IL-1β, IL-6, TNF-α, and chemokine ligand 2. Thus, we should recognize that the PCX-induced neuropathy is a complex pathology that involves the dysfunction of multiple molecules, cells, and organs. Understanding the pathology and treatment of CIPN will be challenging if focusing on only a single molecule. Instead, we need to examine it as a large unit and as a complex phenomenon. For the treatment, Kampo medicines, such as GJG, would be suitable because they include multiple active ingredients and are very complex medicines. Thus, we focused on the activation of S1 astrocytes by PCX and the effect of GJG. GJG inhibited the PCX-induced neuropathy, which was strongly associated with the inhibition of astrocytic activation in the S1 cortex. We previously showed that GJG inhibits oxaliplatin-induced neuropathy, which was accentuated by Bushi (TJ-3023, Tsumura & Co.), a herbal medicine derived from processed aconite root that is included in GJG. We also showed that in mice, Bushi directly acts on inhibits astrocytes, which leads to the suppression of mechanical allodynia. Bushi is not a single chemical; it contains a numerous active molecules that inhibit several types of pains, among which benzo- ylmesaconine, a major alkaloidal compound in Bushi, is considered the main ingredient that inhibits pain via the Na+ channel. Recently, neoline, another ingredient contained in Bushi, was demonstrated to be responsible for inhibiting several types of pains induced by oxaliplatin (a platinum-based anticancer drug), SNL, and PCX. One of the targets of neoline is the Na+ channel, Nav1.7, but its analgesic effect against SNL- or PCX-induced neuropathic pain has a slow onset. In addition, the time course of the analgesic effect of neoline corresponds with that of the inhibitory effect of astrocytes by Bushi in a mouse SNL model, which suggests that neoline inhibits astrocytes. However, it is unclear whether the inhibitory effect of GJG on S1 astrocytes is caused by Bushi or not, and whether the active component in Bushi is neoline or another active molecule. Furthermore, it is not known which specific molecules are regulated by GJG to suppress S1 astrocytes and turn, PCX-induced neuropathy. As mentioned, the pathology of PCX-induced neuropathy is complex and Kampo medicine is also complex. Although we believe that complex pathologies should be treated with complex drugs, further research using big data is needed to uncover the true underlying mechanisms.

Taken together, we demonstrated that treatment with PCX increases the activity of S1 astrocytes, but not S1 microglia, spinal microglia, or spinal astrocytes. The finding that the inhibition of activated S1 astrocytes suppressed the PCX-induced neuropathy strongly suggests that activation of S1 astrocytes is a key feature of the pathogenesis. Although previous studies on CIPN were focused primarily on peripheral neurons and glial cells in the spinal cords, we revealed that S1 astrocytes play a critical role in the regulation of sensory processing. Therefore, they may be offered as a novel target for the prevention of CIPN. The inhibitory effect of GJG and its numerous active ingredients is particularly promising.
ETHICS APPROVAL STATEMENT
All research in this study is conducted with the permission of the University of Yamanashi's Animal Experiment Committee and Genetic Recombination Experiment Committee.

ACKNOWLEDGMENTS
This work was supported by JSPS Grants-in-aid for Scientific Research (KAKENHI) JP25117003, 18H0512, 19H04746, 20H05060, 20H05902, 21H04786, and 21K19309 to S.K.). AMED-CREST (JP20gm1310008 to SK). Takeda Science Foundation (to SK), The Mitsubishi Foundation (to SK), and Frontier in Brain Science Program in Univ Yamanashi (to SK). The authors thank Sarina Iwabuchi, PhD, from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

DISCLOSURE
Goshajinkigan used in this study was provided by Tsumura & Co. (Tokyo, Japan).

PATENT CONSENT STATEMENT
There is no patent for this study.

DATA AVAILABILITY STATEMENT
Data related to this study will be sent by the corresponding author upon reasonable request.

ORCID
Schuichi Koizumi  https://orcid.org/0000-0001-6184-3106

REFERENCES
1. Wilkes G. Peripheral neuropathy related to chemotherapy. Semin Oncol Nurs. 2007;23(3):162-173. DOI: https://doi.org/10.1016/j.soncn.2007.05.001.
2. Flatters SJL, Dougherty PM, Colvin LA. Clinical and preclinical perspectives on Chemotherapy-Induced Peripheral Neuropathy (CIPN): a narrative review. Br J Anaesth. 2017;119(4):737-749. DOI: https://doi.org/10.1093/bja/aex229.
3. Tsuda M, Shigemoto-Mogami Y, Koizumi S, et al. P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. Nature. 2003;424(6950):778-783. DOI: https://doi.org/10.1038/nature01786.
4. Shibata K, Sugawara T, Fujishita K, et al. The astrocyte-targeted therapy by Bushi for the neuropathic pain in mice. PLoS One. 2011;6(8):e23510. DOI: https://doi.org/10.1371/journal.pone.0023510.
5. Kim SK, Hayashi H, Ishikawa T, et al. Cortical astrocytes rewire somatosensory cortical circuits for peripheral neuropathic pain. J Clin Invest. 2016;126(5):1983-1997. DOI: https://doi.org/10.1172/JCI82859.
6. Kim SK, Nabekura J, Koizumi S. Astrocyte-mediated synapse remodeling in the pathological brain. Glia. 2017;65(11):1719-1727. DOI: https://doi.org/10.1002/glia.23169.
7. Kono T, Hata T, Morita S, et al. Goshajinkigan oxaliplatin neurotoxicity evaluation (GONE): a phase 2, multicenter, randomized, double-blind, placebo-controlled trial of Goshajinkigan to prevent oxaliplatin-induced neuropathy. Cancer Chemother Pharmacol. 2013;72(6):1283-1290. DOI: https://doi.org/10.1007/s00280-013-2306-7.
8. Oki E, Emi Y, Kojima H, et al. 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(4):767-775. DOI: https://doi.org/10.1007/s10147-015-0784-9.
9. Bahar MA, Andoh T, Ogura K, Hayakawa Y, Saiki I, Kuraishi Y. Herbal medicine Goshajinkigan prevents paclitaxel-induced mechanical allodynia without impairing antitumor activity of paclitaxel. Evid Based Complement Alternat Med. 2013;2013:849754. DOI: https://doi.org/10.1155/2013/849754.
10. Mizuno K, Shibata K, Komatsu R, Omiya Y, Kase Y, Koizumi S. An effective therapeutic approach for oxaliplatin-induced peripheral neuropathy using a combination therapy with Goshajinkigan and Bushi. Cancer Biol Ther. 2016;17(11):1206-1212. DOI: https://doi.org/10.1080/15384047.2016.1210742.
11. da Costa R, Passos GF, Quintão NLM, Fernandes ES, Maia JRLCB, Campos MM. Taxane-induced neurotoxicity: pathophysiology and therapeutic perspectives. Br J Pharmacol. 2020;177(14):3127-3146. DOI: https://doi.org/10.1111/bph.15086.
12. Tanabe Y, Shiraishi S, Hashimoto K, et al. Taxane-induced sensory peripheral neuropathy is associated with an SCN9A single nucleotide polymorphism in Japanese patients. BMC Cancer. 2020;20:325. DOI: https://doi.org/10.1186/s12885-020-06834-0.
13. Matsumoto M, Inoue M, Hald A, Yamaguchi A, Ueda H. Characterization of three different sensory fibers by use of neocortical capsaicin treatment, spinal antagonism and a novel electrical stimulation-induced paw flexion test. Mol Pain. 2006;2:16. DOI: https://doi.org/10.1186/1744-8069-2-16.
14. Matsumoto M, Inoue M, Hald A, Xie W, Ueda H. Inhibition of paclitaxel-induced A-fiber hypersensitization by gabapentin. J Pharmacol Exp Ther. 2006;318(2):735-740. DOI: https://doi.org/10.1124/jpet.106.103614.
15. Tsuda M, Kohro Y, Yano T, et al. JAK-STAT3 pathway regulates spinal astrocyte proliferation and neuropathic pain maintenance in rats. Brain. 2011;134(4):1127-1139. DOI: https://doi.org/10.1093/brain/awr025.
16. Wang YF, Hatton GI. Astrocytic plasticity and patterned oxytocin neuronal activity: dynamic interactions. J Neurosci. 2009;29(6):1743-1754. DOI: https://doi.org/10.1523/JNEUROSCI.4669-08.2009.
17. Huhnhnchen P, Boehmerle W, Springer A, Freyer D, Endres M. A novel preventive therapy for paclitaxel-induced cognitive deficits: preclinical evidence from C57BL/6 mice. Transl Psychiatry. 2017;7(8):e1185. DOI: https://doi.org/10.1038/tpp.2017.149.
18. Callaghan CK, O'Mara SM. Long-term cognitive dysfunction in the rat following docetaxel treatment is ameliorated by the phosphodiesterase-4 inhibitor, rolipram. Behav Brain Res. 2015;290:84-89. DOI: https://doi.org/10.1016/j.bbr.2015.04.044.
19. Toma W, Kyte SL, Bagdas D, et al. Effects of paclitaxel on the development of neuropathy and affective behaviors in the mouse. Neuropharmacology. 2017;117:305-315. DOI: https://doi.org/10.1016/j.neuropharm.2017.02.020.
20. Shinozaki Y, Shibata K, Yoshida K, et al. Transformation of astrocytes to a neuroprotective phenotype by microglia via P2Y1 receptor downregulation. Cell Rep. 2017;19(6):1151-1164. DOI: https://doi.org/10.1016/j.celrep.2017.04.047.
21. Sano F, Shigetomi E, Shinozaki Y, et al. Reactive astrocyte-driven epileptogenesis is induced by microglia initially activated following status epilepticus. JCI Insight. 2021;6(9):e135391. DOI: https://doi.org/10.1172/jci.insight.135391.
22. Zhang H, Yoon SY, Zhang H, Dougherty PM. Evidence that spinal astrocytes but not microglia contribute to the pathogenesis of Paclitaxel-induced painful neuropathy. J Pain. 2012;13(3):293-303. DOI: https://doi.org/10.1016/j.jpain.2011.12.002.
23. Suzuki T, Miyamoto K, Yokoyama N, et al. Processed aconite root and its active ingredient neoline may alleviate oxaliplatin-induced peripheral neuropathic pain. J Ethnopharmacol. 2016;186:44-52. DOI: https://doi.org/10.1016/j.jep.2016.03.056.
24. Tanimura Y, Yoshida M, Ishiiuchi K, Ohswawa M, Makino T. Neoline is the active ingredient of processed aconite root against murine...
Takanashi K, Shibata K, Mizuno K, Komatsu R, Koizumi S. Goshajinkigan attenuates paclitaxel-induced neuropathic pain via cortical astrocytes. Pharmacol Res Perspect. 2021;9:e00850. https://doi.org/10.1002/prp2.850