The anticancer agents including oxaliplatin, paclitaxel, and bortezomib cause severe peripheral neuropathy. The Kampo medicine Sokeikakketsuto (SOKT) has been widely used to treat several types of pain. In this study, the analgesic effects of SOKT on oxaliplatin-, paclitaxel-, and bortezomib-induced peripheral neuropathy were investigated in rat models. Rats were treated with oxaliplatin (4 mg/kg, intraperitoneally (i.p.), twice a week for four weeks), paclitaxel (4 mg/kg, i.p., twice a week for two weeks), or bortezomib (0.2 mg/kg, i.p., twice a week for two weeks). SOKT (0.3 or 1.0 g/kg) or duloxetine hydrochloride (30 mg/kg, as a positive control) was administered orally after neuropathy developed. Mechanical allodynia and cold hyperalgesia were assessed using the von Frey test and the acetone test, respectively. These tests were performed immediately before and 30, 60, 90, and 120 min after the administration of the drugs. Repeated treatment of oxaliplatin induced mechanical allodynia and cold hyperalgesia. A single administration of SOKT (1 g/kg, per os (p.o.)), as well as duloxetine, temporarily reversed both the mechanical allodynia and the cold hyperalgesia. Repeated administration of paclitaxel and bortezomib also induced the mechanical allodynia. SOKT and duloxetine reversed the mechanical allodynia caused by bortezomib, but not by paclitaxel. SOKT might have the potential to become a new drug to relieve the symptom of oxaliplatin- or bortezomib-induced peripheral neuropathy.

Key words analgesic effect; oxaliplatin; paclitaxel; bortezomib; peripheral neuropathy; Sokeikakketsuto

INTRODUCTION

Although many of the adverse events associated with cancer chemotherapy have become controllable with the supportive cares, peripheral neurotoxicity caused by platinum, taxanes and proteasome inhibitors remains a troubling side effect. These chemotherapeutic agents-induced peripheral neurotoxicity is a dose-limiting adverse event that may necessitate discontinuation or dose reduction. In 2020, the American Society of Clinical Oncology (ASCO) updated a clinical practice guideline for chemotherapy-induced peripheral neuropathy. However, only duloxetine was recommended for the neuropathy treatment in the guideline. Sokeikakketsuto (SOKT), a Kampo medicine, has been used to relieve several acute and chronic pain symptoms, such as arthralgia, neuralgia, low back pain, and myalgia, in Japan. In this study, we investigated whether SOKT has analgesic effects on oxaliplatin-, paclitaxel-, and bortezomib-related neuropathic pain in rats.

MATERIALS AND METHODS

Animals Six-week old male Sprague–Dawley rats (200–250 g, Japan SLC, Inc., Shizuoka, Japan) were divided into groups of four rats per cage and kept under lights from 7:00 to 19:00. The animal experiments were approved by the Experimental Animal Care and Use Committee of Kyushu University.

Drugs Paclitaxel and bortezomib were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Oxaliplatin and SOKT (TJ-53, Lot. 2170053010) were obtained from Yakult Honsha Co., Ltd. (Tokyo, Japan) and Tsumura & Co. (Tokyo, Japan), respectively. SOKT extract (5.0 g) is a dried extract of the following mixed crude drugs (total 27.5 g): Paeoniae Radix (2.5 g), Rehmaniae Radix (2.0 g), Gentianae Scabrae Radix (1.0 g), Angelicae Dahuricae Radix (1.5 g), Atractylodes Rhizome (2.0 g), Atractylodes Rhizome (1.5 g), Sinomeni Caulis et Rhizoma (1.5 g), Gentiana Scabrae Radix (1.5 g), Glycyrrhiza Radix (1.0 g), Angelicae Dahuricae Radix (1.0 g), and Zingiberis Rhizoma (0.5 g). Each plant material was identified by its external morphology and authenticated by marker compounds of plant specimens according to the methods of the Japanese Pharmacopoeia. To produce the dried SOKT extract powder, the mixture of seventeen component herbs was extracted with purified hot water at 95°C for 1 h. The extract solution was separated from the insoluble waste, and concentrated by removing water under reduced pressure. Spray drying was used to produce the dried extract powder. Duloxetine hydrochloride was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Oxaliplatin (4 mg/kg) or a vehicle (5% glucose solution) was intraperitoneally (i.p.) injected on days 1, 2, 8, 9, 15, 16, 22, and 23. To determine the analgesic effects of SOKT and duloxetine, as a positive control, on oxaliplatin-induced mechanical allodynia and cold hyperalgesia, behavioral tests were performed immediately before and 30, 60, 90, and 120 min after the administration of the drugs. Repeated treatment of oxaliplatin induced mechanical allodynia and cold hyperalgesia. A single administration of SOKT (1 g/kg, per os (p.o.)), as well as duloxetine, temporarily reversed both the mechanical allodynia and the cold hyperalgesia. Repeated administration of paclitaxel and bortezomib also induced the mechanical allodynia. SOKT and duloxetine reversed the mechanical allodynia caused by bortezomib, but not by paclitaxel. SOKT might have the potential to become a new drug to relieve the symptom of oxaliplatin- or bortezomib-induced peripheral neuropathy.

Key words analgesic effect; oxaliplatin; paclitaxel; bortezomib; peripheral neuropathy; Sokeikakketsuto

* To whom correspondence should be addressed. e-mail: tkawa@med.kyushu-u.ac.jp; kawashiri.takehiro.650@m.kyushu-u.ac.jp

© 2021 The Pharmaceutical Society of Japan
were assessed on days 24 and 25 after neuropathy developed. Paclitaxel (4 mg/kg, i.p.) or a vehicle (50% ethanol and 50% Kolliphor EL (Sigma-Aldrich Co., MO, U.S.A.)) was injected on days 1, 4, 8, and 11. Bortezomib (0.2 mg/kg, i.p.) or a vehicle (saline) was treated on the same schedule. To determine the effects of SOKT and duloxetine on paclitaxel and bortezomib-induced mechanical allodynia, the tests were performed on days 12 and 13. SOKT extract (0.3 or 1.0 g/kg), duloxetine hydrochloride (30 mg/kg) or a vehicle (distilled water) was treated orally, and behavioral tests were assessed immediately before and 30–120 min after drug treatment. The clinical dose of SOKT is about 0.1 g/kg/d in human. Considering species differences, 0.3 and 1 g/kg were used. For reference, 0.3 and 1 g/kg of Goshajinkigan have been used in animal studies, although the clinical dose of it is also about 0.1 g/kg/d.

**Behavioral Tests** The method for evaluating mechanical allodynia in the von Frey test has been described in a previous literature. Von Frey filaments (Aesthesio®; DanMic Global, LLC, San Jose, CA, U.S.A.) were applied to the mid-plantar of each hind paw for 6 s, and the withdrawal threshold was determined. The acetone test was performed to assess cold hyperalgesia as described previously. The number of withdrawal responses was counted for 40 s after acetone was sprayed on the plantar skin.

**Statistical Analyses** Data were presented as the mean ± standard error of the mean. Statistical analyses were performed by the Student’s *t*-test or two-way ANOVA followed by the Tukey–Kramer test to determine the differences between groups (StatView; Abacus Concepts, CA, U.S.A.). *p* < 0.05 was considered statistically significant.

**RESULTS**

Repeated treatment with oxaliplatin reduced the withdrawal threshold compared with the vehicle in the von Frey test (*p* < 0.01, Figs. 1A, B). Both SOKT (1.0 g/kg *per os* (*p.o.*)) and duloxetine hydrochloride (30 mg/kg) almost completely reversed the oxaliplatin-caused reduction in the withdrawal threshold 30 min after the administrations (*p* < 0.01, Figs. 1A, B). The effects of both SOKT and duloxetine disappeared by 120 min after the administrations. In the acetone tests, oxaliplatin also increased the number of withdrawal responses to cold stimulation (*p* < 0.01, Figs. 1C, D). Both SOKT and duloxetine almost completely reversed the oxaliplatin-induced increase in withdrawal responses 30 min after the administration (SOKT, *p* < 0.01; duloxetine, *p* < 0.05, Figs. 1C, D). The effects of the drugs disappeared by 120 min after the administrations.

Administrations of paclitaxel reduced the pain threshold in the von Frey test (*p* < 0.01, data not shown). Neither SOKT nor duloxetine reversed the paclitaxel-induced reduction in the threshold (data not shown).

Injections of bortezomib reduced the threshold in the von Frey test (*p* < 0.01, Figs. 2A, B). Both SOKT and duloxetine almost completely reversed the reduction in the threshold 30 min after the administration (*p* < 0.01, Figs. 2A, B).

**DISCUSSION**

The data in this study revealed that a single administration of SOKT and duloxetine completely reversed oxaliplatin-induced mechanical allodynia and cold hyperalgesia, and bortezomib-induced mechanical allodynia, but not paclitaxel-induced mechanical allodynia in rat models. Thus, SOKT...
many side adverse effects such as nausea, headache, somnolence, sexual dysfunction, dry mouth and dizziness effects.\textsuperscript{18} Hence, it is considered safer to use SOKT than duloxetine.

In conclusion, this study demonstrates for the first time that SOKT ameliorates peripheral neuropathy symptoms induced by oxaliplatin and bortezomib in rats. Therefore, SOKT is expected to be useful as symptomatic therapy for clinical chemotherapy-induced peripheral neuropathy.

\textbf{Acknowledgments} We are grateful to Tsumura & Co. for providing SOKT for this study. This work was partly supported by JSPS KAKENHI (20K07198).

\textbf{Conflict of Interest} The authors declare no conflict of interest.

\textbf{Supplementary Materials} The online version of this article contains supplementary materials.

\textbf{REFERENCES}

1) Sałat K. Chemotherapy-induced peripheral neuropathy: part 1-current state of knowledge and perspectives for pharmacotherapy. \textit{Pharmacol. Rep.}, 72, 486–507 (2020).

2) Loprinzi CL, Lacchetti C, Bleeker I, Cavalletti G, Chauhan C, Hertz DL, Kelley MR, Lavino A, Lustberg MB, Paice JA, Schneider BP, Lavoie Smith EM, Smith ML, Smith TJ, Wagerer-Ashton N, Hershman DL. Prevention and management of chemotherapy-induced peripheral neuropathy in survivors of adult cancers: ASCO guideline update. \textit{J. Clin. Oncol.}, 38, 3325–3348 (2020).

3) Iwanga K, Hayashi M, Hamahata Y, Miyazaki M, Shibano M, Taniguchi M, Baba K, Kakemi M. Furanocoumarin derivatives in Kampo extract medicines inhibit cytochrome P450 3A4 and P-glycoprotein. \textit{Drug Metab. Dispos.}, 38, 1286–1294 (2010).

4) Ushio S, Egashira N, Sada H, Kawashiri T, Shirahama M, Masuguchi K, Oishi R. Goshajinkigan reduces bortezomib-induced mechanical allodynia in rats: possible involvement of kappa opioid receptor. \textit{J. Pharmacol. Sci.}, 129, 196–199 (2015).

5) Kawashiri T, Egashira N, Watanabe H, Ikeyami Y, Hirakawa S, Mihara Y, Yano T, Ikuse H, Oishi R. Prevention of oxaliplatin-induced mechanical allodynia and neurodegeneration by neurotropin in the rat model. \textit{Eur. J. Pain}, 15, 344–350 (2011).

6) Sakurai M, Egashira N, Kawashiri T, Yano T, Ikuse H, Oishi R. Oxaliplatin-induced neuropathy in the rat: involvement of oxalate in cold hyperalgesia but not mechanical allodynia. \textit{Pain}, 147, 165–174 (2009).

7) Smith EM, Pang H, Circrione 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. \textit{JAMA}, 309, 1359–1367 (2013).

8) Tatsushima Y, Egashira N, Kawashiri T, Mihara Y, Yano T, Mishima K, Oishi R. Involvement of substance P in peripheral neuropathy induced by paclitaxel but not oxaliplatin. \textit{J. Pharmacol. Exp. Ther.}, 337, 226–235 (2011).

9) Yamamoto S, Ushio S, Egashira N, Kawashiri T, Mitsuwasu S, Higuchi H, Ozawa N, Masuguchi K, Ono Y, Masuda S. Excessive spinal glutamate transmission is involved in oxaliplatin-induced mechanical allodynia: a possibility for riluzole as a prophylactic drug.
11) Mihara Y, Egashira N, Sada H, Kawashiri T, Ushio S, Yano T, Ikesue H, Oishi R. Involvement of spinal NR2B-containing NMDA receptors in oxaliplatin-induced mechanical allodynia in rats. Mol. Pain, 7, 1744-8069-7-8 (2011).

12) Zhang XJ, Chen HL, Li Z, Zhang HQ, Xu HX, Sung JJ, Bian ZX. Analgesic effect of paeoniflorin in rats with neonatal maternal separation-induced visceral hyperalgesia is mediated through adenosine A1 receptor by inhibiting the extracellular signal-regulated protein kinase (ERK) pathway. Pharmacol. Biochem. Behav., 94, 88–97 (2009).

13) Kawakami Z, Ikarashi Y, Kase Y. Isoliquiritigenin is a novel NMDA receptor antagonist in kampo medicine yoku kansan. Cell Mol. Neurobiol., 31, 1203–1212 (2011).

14) Andoh T, Kobayashi N, Uta D, Kuraishi Y. Prophylactic topical paeoniflorin prevents mechanical allodynia caused by paclitaxel in mice through adenosine A1 receptors. Phytomedicine, 25, 1–7 (2017).

15) Andoh T, Uta D, Kato M, Toume K, Komatsu K, Kuraishi Y. Prophylactic administration of aucubin inhibits paclitaxel-induced mechanical allodynia via the inhibition of endoplasmic reticulum stress in peripheral Schwann cells. Biol. Pharm. Bull., 40, 473–478 (2017).

16) 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, 849754 (2013).

17) Matsumura Y, Yokoyama Y, Hirakawa H, Shigeto T, Futagami M, Mizunuma H. The prophylactic effects of a traditional Japanese medicine, goshajinkigan, on paclitaxel-induced peripheral neuropathy and its mechanism of action. Mol. Pain, 10, 1744-8069-10-61 (2014).

18) Bitter I, Filipovits D, Czobor P. Adverse reactions to duloxetine in depression. Expert Opin. Drug Saf., 10, 839–850 (2011).