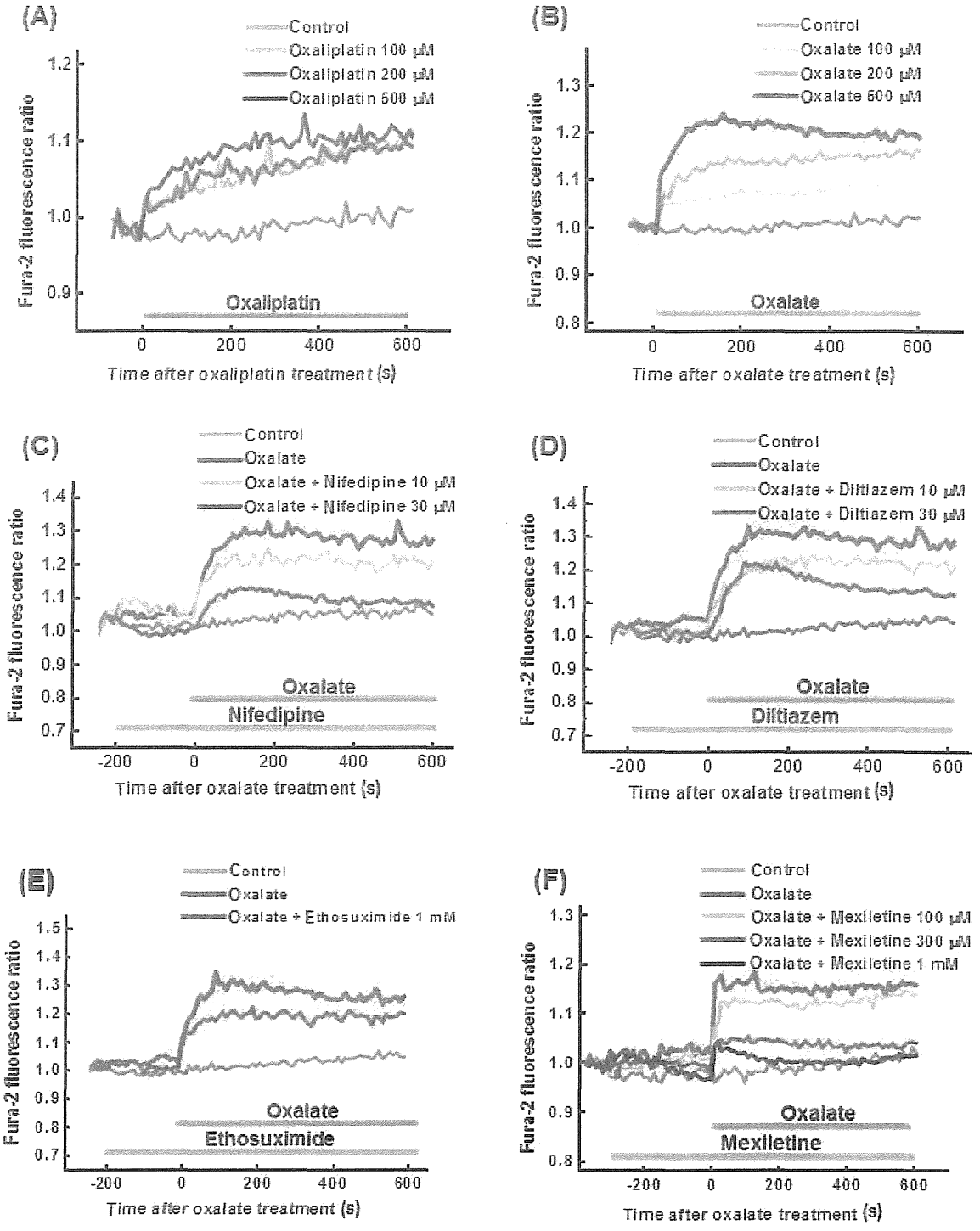

Figure 2 Increase in the intracellular Ca^{2+} following oxaliplatin or oxalate treatment in primary cultured DRG cells. Oxaliplatin (**A**: 100-500 μM) or sodium oxalate (**B**: 100-500 μM) was administered to cultured DRG cells. Nifedipine (**C**: 10-30 μM), diltiazem (**D**: 10-30 μM), ethosuximide (**E**: 1 mM) or mexiletine (**F**: 100 μ-1 mM) were co-administered with sodium oxalate (500 μM) to cells. Intracellular Ca^{2+} levels were determined based on Fura-2 fluorescence (340 nm/380 nm). Values are expressed as the mean of 4-8 wells.

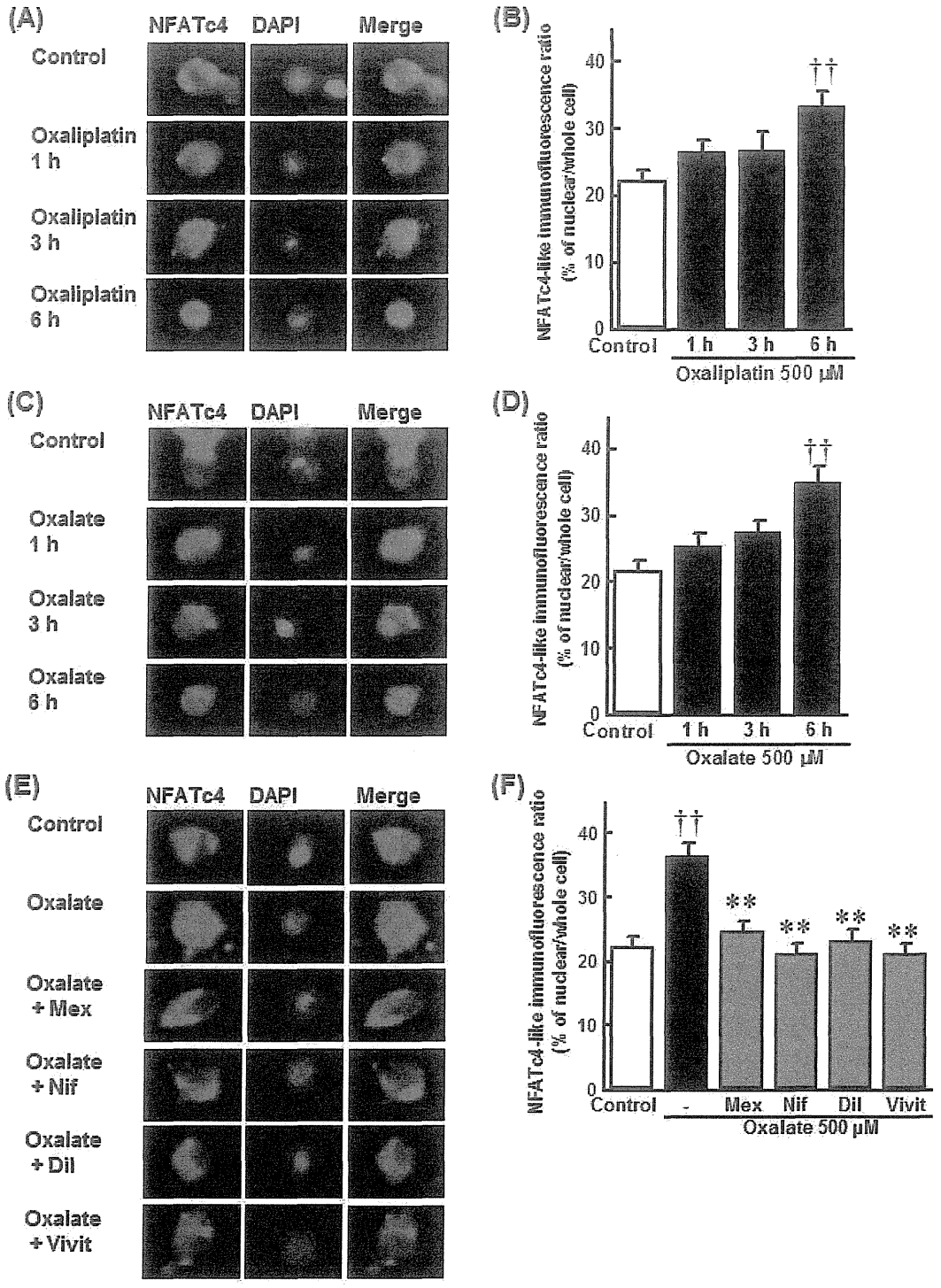

Figure 3 NFAT nuclear translocation in primary cultured DRG cells. Oxaliplatin (**A**, **B**: 500 μM for 1-6 h) or sodium oxalate (**C**, **D**: 500 μM for 1-6 h) was administered to cultured DRG cells. **E**, **F**: Mexiletine (Mex, 1 mM), nifedipine (Nif, 30 μM), diltiazem (Dil, 30 μM) or vivit (2 μM) was co-administered with sodium oxalate (500 μM) to cells for 6 h. NFATc4 immunostaining (green) and nuclear staining with DAPI (blue). NFATc4 and DAPI-positive nuclei were visualized by fluorescence microscopy (A, C, E). The nuclear translocation of NFATc4 was calculated by comparing the ratio of nuclear NFATc4 immunofluorescence/total NFATc4 immunofluorescence (B, D, F). Values are expressed as the mean ± SEM of 24-33 cells. ††$p < 0.01$ compared with control group, **$p < 0.01$ compared with oxalate group.

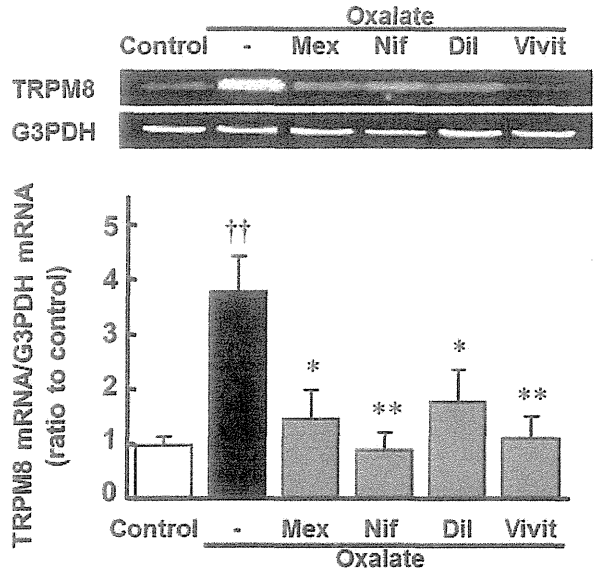

Figure 4 Reversal of the oxalate-induced increase in TRPM8 mRNA in cultured DRG cells by Ca^{2+} and Na^{+} channel blockers. Mexiletine (Mex, 1 mM), nifedipine (Nif, 30 μM), diltiazem (Dil, 30 μM) or vivit (2 μM) was co-administered with sodium oxalate (500 μM) to cells for 12 h. The mRNA expression of TRPM8 and G3PDH were determined by PCR. Values are expressed as the mean ± SEM of 6 wells. ††$p < 0.01$ compared with control group, *$p < 0.05$, **$p < 0.01$ compared with oxalate group.

and diltiazem: $p < 0.05$; nifedipine: $p < 0.01$). Similarly, vivit (2 μM) completely reversed the oxalate-induced increase in TRPM8 mRNA levels ($p < 0.01$).

Ca^{2+} and Na^{+} channel blockers inhibit the oxaliplatin-induced cold hyperalgesia and increase in TRPM8 mRNA levels in the DRG in rats

Co-administration with nifedipine (10, 30 mg/kg, p.o.) completely inhibited the oxaliplatin-induced increase in withdrawal responses to acetone spray in rats (Figure 5A, $p < 0.01$). Diltiazem (10, 30 mg/kg, p.o.) also strongly inhibited the oxaliplatin-induced increase in withdrawal responses (Figure 5B, $p < 0.01$). Similarly, mexiletine (10, 30 mg/kg, p.o.) attenuated the oxaliplatin-induced increase in withdrawal responses (Figure 5D, $p < 0.01$). By contrast, ethosuximide (300 mg/kg, p.o.) only weakly prevented the oxaliplatin-induced increase in withdrawal responses (Figure 5C, days 3 and 8: $p < 0.05$). Moreover, co-administration with mexiletine (30 mg/kg, p.o.), nifedipine (30 mg/kg, p.o.) or diltiazem (30 mg/kg, p.o.) completely inhibited the oxaliplatin-induced increase in TRPM8 mRNA levels on day 5 (Figure 6, $p < 0.01$).

Discussion

Oxaliplatin was previously reported to induce cold allodynia and increase in TRPM8 mRNA levels in the DRG after 3 days in mice [16] and increase the TRPM8 mRNA levels in cultured rat DRG cells [19]. Consistent with these reports, in the present study we demonstrated that oxaliplatin induced cold hyperalgesia in rats on days 3, 5 and 8 and increased the TRPM8 mRNA levels in the DRG on day 5, the peak of cold hyperalgesia. Furthermore, we found that oxalate significantly induced the increase in TRPM8 protein in the DRG on day 5. In addition, we confirmed that oxaliplatin markedly increased the TRPM8 mRNA levels in primary cultured DRG cells.

TRPM8 is known to be involved in cold sensitivity [20] and cold allodynia after chronic nerve injury [21]. Moreover, TRPM8-deficient mice attenuate behavioral response to cold stimulation [22,23]. Oxaliplatin-induced cold allodynia is reversed by capsazepine, a blocker of both TRPM8 and TRP vanilloid 1 (TRPV1), but not by 5'-iodoresiniferatoxin, a selective TRPV1 blocker [16]. Hence, the increase in TRPM8 expression in DRG neurons may be involved in oxaliplatin-induced cold hyperalgesia. Recently, Nassini et al. [24] have reported that oxaliplatin induces mechanical and cold allodynia via TRP ankyrin 1 (TRPA1) activation in rodents. Considering these collective findings, both up-regulation of TRPM8 and activation of TRPA1 may be involved in the cold hypersensitivity by oxaliplatin. We also found that treatment with oxalate, a metabolite of oxaliplatin, markedly increased the TRPM8 mRNA levels in primary cultured DRG cells. Furthermore, oxalate significantly induced the increase in TRPM8 protein in the DRG. Oxaliplatin is rapidly metabolized to $Pt(dach)Cl_2$ in rat blood *in vitro* [25], suggesting that oxalate is immediately derived from oxaliplatin. We previously reported that oxalate induced cold hyperalgesia/allodynia but not mechanical allodynia in rats [7]. Taken together, these data suggest that oxalate may be involved in the oxaliplatin-induced increase in TRPM8 expression, resulting in cold hyperalgesia.

In the present study, both oxaliplatin and oxalate increased the intracellular Ca^{2+} levels in primary cultured DRG cells, and the oxalate-induced increase in intracellular Ca^{2+} level was inhibited by nifedipine (an L type Ca^{2+} channel blocker) and diltiazem (an L/T type Ca^{2+} channel blocker). By contrast, ethosuximide (a T type Ca^{2+} channel blocker) only weakly attenuated the oxalate-induced increase in intracellular Ca^{2+}. Thus, it is likely that oxaliplatin induces Ca^{2+} influx via mainly L type Ca^{2+} channels. Oxaliplatin was reported to increase the amplitude and duration of compound action potentials interacting with voltage-gated Na^{+} channels in rat sensory neurons [9], and prolong the duration of the A-fiber compound action potential related to K^{+} channels [12]. Thus, enhancement of action potentials via Na^{+} or K^{+} channels might result in Ca^{2+} influx through L type Ca^{2+} channels. This mechanism is supported by the

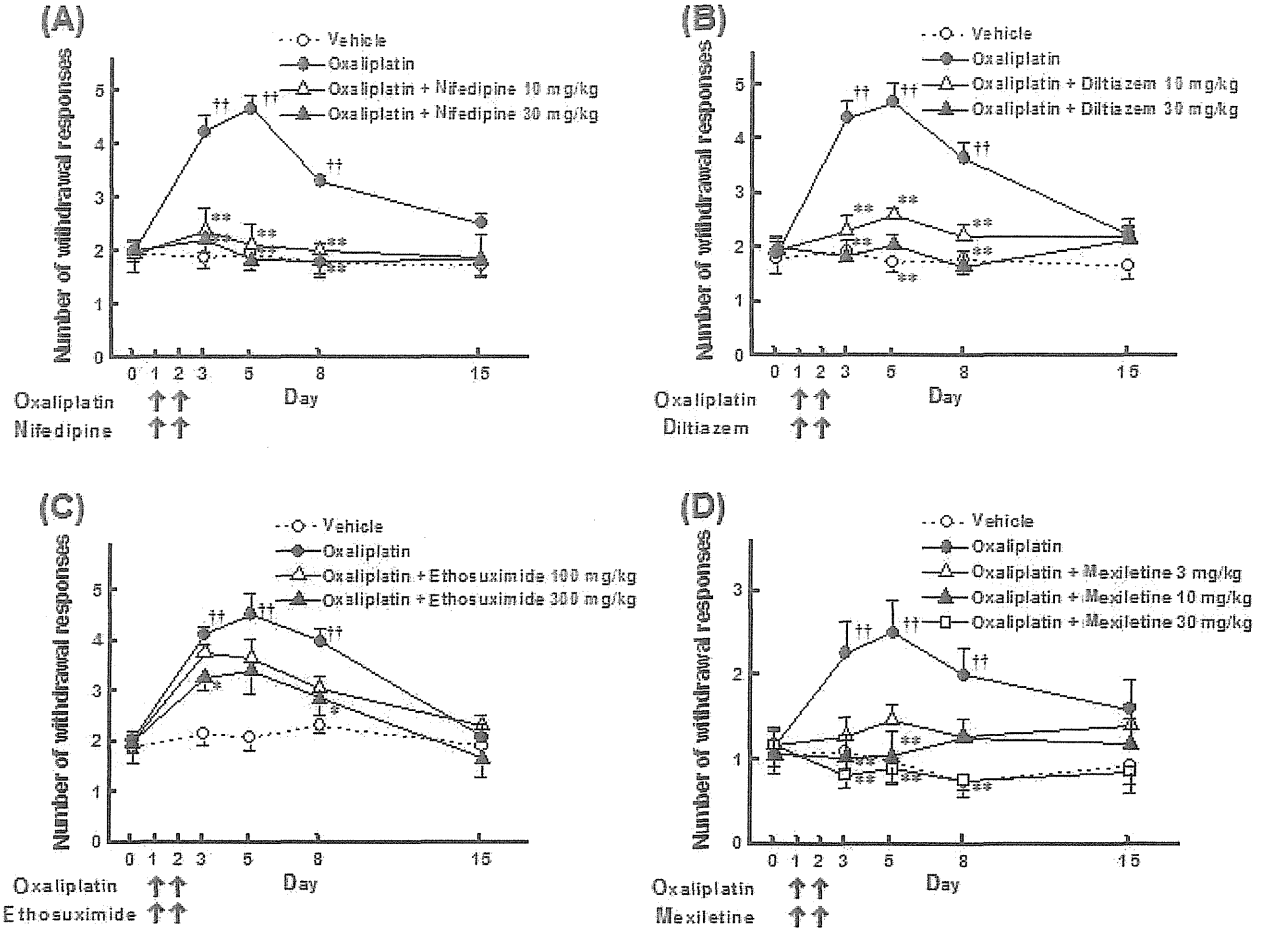

Figure 5 Reversal of the oxaliplatin-induced cold hyperalgesia in rats by Ca^{2+} and Na^{+} channel blockers. Oxaliplatin (4 mg/kg) was administered i.p. on days 1 and 2. Nifedipine (**A**: 10 and 30 mg/kg), diltiazem (**B**: 10 and 30 mg/kg), ethosuximide (**C**: 100 and 300 mg/kg) or mexiletine (**D**: 3-30 mg/kg) was orally co-administered with oxaliplatin. Acetone test was performed on days 0, 3, 5, 8 and 15. Values are expressed as the mean ± SEM of 6-10 animals. ††$p < 0.01$ compared with vehicle group, *$p < 0.05$, **$p < 0.01$ compared with oxaliplatin group.

present result that the Na^{+} channel blocker mexiletine completely reversed the oxalate-induced Ca^{2+} influx.

In general, NFAT is activated and translocated into the nucleus via Ca^{2+} signaling [26]. In the present study, both oxaliplatin and oxalate induced the nuclear translocation of NFAT in cultured DRG cells, and the oxalate-induced NFAT nuclear translocation was completely blocked by nifedipine, diltiazem and mexiletine, as well as vivit, a selective NFAT inhibitor. Furthermore, nifedipine, diltiazem, mexiletine and vivit reversed the oxalate-induced increase in TRPM8 mRNA levels in cultured DRG cells. Taken together, these data suggest that oxalate may induce up-regulation of TRPM8 expression via NFAT activation by Ca^{2+} influx through L/T type Ca^{2+} channels derived from Na^{+} channels activation. We also confirmed that co-administration with nifedipine, diltiazem or mexiletine inhibited the oxaliplatin-induced cold hyperalgesia and increase in TRPM8 mRNA levels in the DRG *in vivo* in rats. Thus, the oxaliplatin-induced cold hyperalgesia is mediated by up-regulation of TRPM8 expression via Na^{+} and Ca^{2+} influx.

In addition, Fajardo et al. [27] have reported that L-type Ca^{2+} channel blockers 1,4-dihydropyridines such as nifedipine activate TRPA1-mediated currents in CHO cells in electrophysiological study. However, they reported that no signs of behavioral pain were observed following local application of nifedipine to the hind paw of mice. Because nifedipine blocks electrically evoked Ca^{2+} transients in peripheral sensory nerves [28], it is possible that these potent inhibitory actions on L-type Ca^{2+} channels prevent the propagation of electrical impulses at nerve terminals, despite a powerful TRPA1 activation.

Conclusions

We demonstrated that L type Ca^{2+} channel/NFAT/TRPM8 pathway plays a crucial role in signaling the

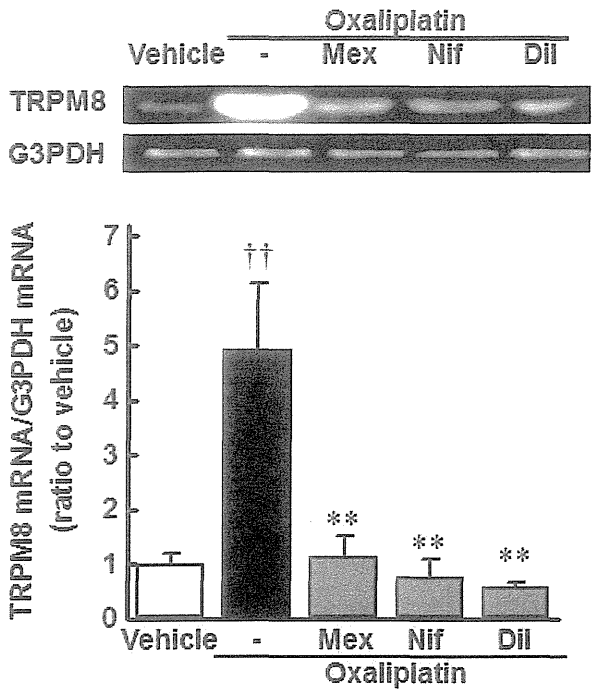

***Figure 6* Reversal of the oxaliplatin-induced increase of TRPM8 mRNA in rat DRG neurons by Ca^{2+} and Na^{+} channel blockers.** Oxaliplatin (4 mg/kg) was administered i.p. on days 1 and 2. Mexiletine (Mex, 30 mg/kg), nifedipine (Nif, 30 mg/kg) or diltiazem (Dil, 30 mg/kg) was orally co-administered with oxaliplatin. The expression of TRPM8 and G3PDH mRNAs were determined by PCR on day 5. Values are expressed as the mean ± SEM of 5 animals. $††p < 0.01$ compared with vehicle group, $**p < 0.01$ compared with oxaliplatin group.

oxaliplatin-induced cold hyperalgesia. Co-administration of L type Ca^{2+} channel blockers inhibited the oxaliplatin-induced cold hyperalgesia. Therefore, novel strategies involving Ca^{2+} channel blockers may be useful for prevention of oxaliplatin-induced acute neuropathy.

Acknowledgements
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Authors' contributions
TK, NE and RO are responsible for experimental design. TK and KK are responsible for performance of behavioral test. TK, KK, KT and YY are responsible for measurement of intracellular Ca^{2+} level, immunostaining and PCR. KT, SU and TY are responsible for performance of Western blotting. TK, NE and RO are responsible for writing the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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References

1. Cassidy J, Misset JL: **Oxaliplatin-related side effects: characteristics and management.** *Semin Oncol* 2002, **29**:11-20.
2. Extra JM, Marty M, Brienza S, Misset JL: **Pharmacokinetics and safety profile of oxaliplatin.** *Semin Oncol* 1998, **25**:13-22.
3. Pasetto LM, D'Andrea MR, Rossi E, Monfardini S: **Oxaliplatin-related neurotoxicity: how and why?** *Crit Rev Oncol Hematol* 2006, **59**:159-168.
4. Quasthoff S, Hartung HP: **Chemotherapy-induced peripheral neuropathy.** *J Neurol* 2002, **249**:9-17.
5. de Gramont A, Vignoud J, Tournigand C: **Oxalipaltin/5FU/LV in adjuvant colon cancer: safety results of the international randomized MOSAIC trial.** *Proc Am Soc Clin Oncol* 2002, **21**:132.
6. Wilson RH, Lehky T, Thomas RR, Quinn MG, Floeter MK, Grem JL: **Acute oxaliplatin-induced peripheral nerve hyperexcitability.** *J Clin Oncol* 2002, **20**:1767-1774.
7. Sakurai M, Egashira N, Kawashiri T, Yano T, Ikesue H, Oishi R: **Oxaliplatin-induced neuropathy in the rat: involvement of oxalate in cold hyperalgesia but not mechanical allodynia.** *Pain* 2009, **147**:165-174.
8. Graham MA, Lockwood GF, Greenslade D, Brienza S, Bayssas M, Gamelin E: **Clinical pharmacokinetics of oxaliplatin: a critical review.** *Clin Cancer Res* 2000, **6**:1205-1218.
9. Adelsberger H, Quasthoff S, Grosskreutz J, Lepier A, Eckel F, Lersch C: **The chemotherapeutic oxaliplatin alters voltage-gated Na^{+} channel kinetics on rat sensory neurons.** *Eur J Pharmacol* 2000, **406**:25-32.
10. Grolleau F, Gamelin L, Boisdron-Celle M, Lapied B, Pelhate M, Gamelin E: **A possible explanation for a neurotoxic effect of the anticancer agent oxaliplatin on neuronal voltage-gated sodium channels.** *J Neurophysiol* 2001, **85**:2293-2297.
11. Kagiava A, Tsingotjidou A, Emmanouilides C, Theophilidis G: **The effects of oxaliplatin, an anticancer drug, on potassium channels of the peripheral myelinated nerve fibres of the adult rat.** *Neurotoxicology* 2008, **29**:1100-1106.
12. Sittl R, Carr RW, Fleckenstein J, Grafe P: **Enhancement of axonal potassium conductance reduces nerve hyperexcitability in an in vitro model of oxaliplatin-induced acute neuropathy.** *Neurotoxicology* 2010, **31**:694-700.
13. Saif MW, Reardon J: **Management of oxaliplatin-induced peripheral neuropathy.** *Ther Clin Risk Manage* 2005, **1**:249-258.
14. McKemy DD, Neuhausser WM, Julius D: **Identification of a cold receptor reveals a general role for TRP channels in thermosensation.** *Nature* 2002, **416**:52-58.
15. Peier AM, Moqrich A, Hergarden AC, Reeve AJ, Andersson DA, Story GM, Earley TJ, Dragoni I, McIntyre P, Bevan S, Patapoutian A: **A TRP channel that senses cold stimuli and menthol.** *Cell* 2002, **108**:705-715.
16. Gauchan P, Andoh T, Kato A, Kuraishi Y: **Involvement of increased expression of transient receptor potential melastatin 8 in oxaliplatin-induced cold allodynia in mice.** *Neurosci Lett* 2009, **458**:93-95.
17. Wang C, Li JF, Zhao L, Liu J, Wan J, Wang YX, Wang J, Wang C: **Inhibition of SOC/Ca^{2+}/NFAT pathway is involved in the anti-proliferative effect of sildenafil on pulmonary artery smooth muscle cells.** *Respir Res* 2009, **10**:123.
18. Zimmermann M: **Ethical guidelines for investigations of experimental pain in conscious animals.** *Pain* 1983, **16**:109-110.
19. Ta LE, Bieber AJ, Carlton SM, Loprinzi CL, Low PA, Windebank AJ: **Transient Receptor Potential Vanilloid 1 is essential for cisplatin-induced heat hyperalgesia in mice.** *Mol Pain* 2010, **6**:15.
20. Bautista DM, Siemens J, Glazer JM, Tsuruda PR, Basbaum AI, Stucky CL, Jordt SE, Julius D: **The menthol receptor TRPM8 is the principal detector of environmental cold.** *Nature* 2007, **448**:204-208.
21. Xing H, Chen M, Ling J, Tan W, Gu JG: **TRPM8 mechanism of cold allodynia after chronic nerve injury.** *J Neurosci* 2007, **27**:13680-13690.
22. Colburn RW, Lubin ML, Stone DJ Jr, Wang Y, Lawrence D, D'Andrea MR, Brandt MR, Liu Y, Flores CM, Qin N: **Attenuated cold sensitivity in TRPM8 null mice.** *Neuron* 2007, **54**:379-386.
23. Dhaka A, Murray AN, Mathur J, Earley TJ, Petrus MJ, Patapoutian A: **TRPM8 is required for cold sensation in mice.** *Neuron* 2007, **54**:371-378.
24. Nassini R, Gees M, Harrison S, De Siena G, Materazzi S, Moretto N, Failli P, Preti D, Marchetti N, Cavazzini A, Mancini F, Pedretti P, Nilius B, Patacchini R, Geppetti P: **Oxaliplatin elicits mechanical and cold allodynia in rodents via TRPA1 receptor stimulation.** *Pain* 2011, **152**:1621-1631.
25. Luo FR, Wyrick SD, Chaney SG: **Biotransformations of oxaliplatin in rat blood in vitro.** *J Biochem Mol Toxicol* 1999, **13**:159-169.

26. Hogan PG, Chen L, Nardone J, Rao A: **Transcriptional regulation by calcium, calcineurin, and NFAT.** *Genes Dev* 2003, 17:2205-2232.
27. Fajardo O, Meseguer V, Belmonte C, Viana F: **TRPA1 channels: novel targets of 1,4-dihydropyridines.** *Channels (Austin)* 2008, 2:429-438.
28. Gover TD, Kao JP, Weinreich D: **Calcium signaling in single peripheral sensory nerve terminals.** *J Neurosci* 2003, 23:4793-4797.

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Clinical and Experimental Gastroenterology

ORIGINAL RESEARCH

Rikkunshito, a traditional Japanese medicine, suppresses cisplatin-induced anorexia in humans

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Background: The aim of this study was to investigate the effects of Rikkunshito on ghrelin secretion and on cisplatin-induced anorexia in humans.

Methods: The study was performed as a crossover design, and ten unresectable or relapsed gastric cancer patients were randomly divided into two groups. Group A (n = 5) was started on Rikkunshito (2.5 g three times daily, orally) from the first course of chemotherapy and followed by a second course without Rikkunshito. A treatment with reversed order was performed for Group B (n = 5). All patients received combined chemotherapy with S-1 plus cisplatin. The primary endpoint was the amount of oral intake, and the categories of scales of anorexia, nausea, and vomiting; secondary endpoints included the plasma concentration of acylated ghrelin.

Results: In the Rikkunshito-on period, no decrease of the plasma concentration of acylated ghrelin induced by cisplatin was observed. The average oral intake in the Rikkunshito-on period was significantly larger than that in the Rikkunshito-off period, and the grade of anorexia was significantly lower in the Rikkunshito-on period than in the Rikkunshito-off period.

Conclusion: Rikkunshito appeared to prevent anorexia induced by cisplatin, resulting in effective prophylactic administration of chemotherapy with cisplatin, and patients could continue their treatments on schedule.

Keywords: Rikkunshito, cisplatin, ghrelin, anorexia, stomach cancer

Introduction

Combined chemotherapy with S-1 plus cisplatin is an attractive chemotherapy regimen for gastric cancer. A previous Phase I/II trial of this regimen in metastatic gastric cancer reported a high response rate of 76% and acceptable toxicity.[1] Recently, a Phase III Japanese trial of chemotherapeutic regimens for metastatic gastric cancer (SPIRITS trial) demonstrated that S-1 plus cisplatin led to significantly longer median overall survival than S-1 alone (13 months versus 11 months).[2] Cisplatin is widely used in various chemotherapies, but undesirable side effects, such as nausea, vomiting, and anorexia, markedly affect the quality of life of patients and may make the continuation of chemotherapy difficult. While some antiemetic agents have been introduced as treatment for nausea and vomiting,[3,4] appetite loss is still present in many cancer patients. However, the mechanism of the resulting appetite loss during chemotherapy is not thoroughly understood.

Ghrelin is an endogenous ligand of the growth hormone secretagogue receptor; it consists of 28 amino acids and is secreted mainly from the stomach.[5] Ghrelin is known to have an intense appetite-enhancing effect in addition to a growth hormone secretion-promoting effect.[6] Ghrelin is the only hormone that exhibits an orexigenic

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effect following peripheral administration.[7] In addition, ghrelin exhibits a variety of actions, including stimulation of growth hormone secretion, gastric motility, and gastric acid secretion,[8] as well as induction of a positive energy balance.[9] The level of plasma ghrelin is thought to be related to gastrointestinal disorders, and ghrelin has been administered to patients with anorexia-related disorders as a new therapy.[10] However, the intravenous and repeated administration of ghrelin presented a considerable burden for the patients.

Rikkunshito, a traditional Japanese medicine, is used to treat various gastrointestinal tract disorders, such as functional dyspepsia, gastroesophageal reflux, dyspeptic symptoms of post-gastrointestinal surgery, and chemotherapy-induced nausea.[11–13] Takeda et al[14] showed that a flavonoid in Rikkunshito suppressed a cisplatin-induced decrease in plasma acylated ghrelin levels and increased food intake in rats, and was mediated by serotonin (5-HT_{2B} and 5-HT_{2C}) receptors. The aim of this study was to investigate the effect of Rikkunshito on ghrelin secretion and on cisplatin-induced anorexia in humans.

Materials and methods

Patient eligibility

Eligible patients had histologically proved unresectable or recurrent gastric cancer. Up to one regimen of prior chemotherapy was allowed (adjuvant chemotherapy was allowed provided that at least 28 days had elapsed since the last treatment), except for prior treatment with cisplatin.

Other inclusion criteria were as follows: age 20–75 years; Eastern Clinical Oncology Group performance status 0–1; estimated life expectancy more than 3 months; a white blood cell count between 4000 and 12,000 mm^3; an absolute neutrophil count of over 2000 mm^3, a platelet count of over 100,000 mm^3, and a hemoglobin level of over 8.0 g/dL; aspartate aminotransferase and alanine aminotransferase levels within two times the upper limit of normal for the institution; serum bilirubin level less than 1.5 mg/dL; serum creatinine level within the upper limit of the normal value for the institution; 24-hour creatinine clearance more than 50 mL/minute; and a normal electrocardiogram. Only patients who could swallow tablets were eligible. Patients were excluded if they had brain metastases, severe comorbid conditions, active double cancers, or a past history of drug allergy or were unable to comply with the protocol requirements. Pregnant women were also excluded. Written informed consent was obtained from all patients before study entry.

Treatment design

The study was performed as a crossover design because of the limited number of patients (Figure 1). Ten patients were randomly divided into two groups. The gender, age, tumor characteristics, and performance status of these 10 patients are listed in Table 1. Group A (n = 5) was started on Rikkunshito from the first course of chemotherapy followed by a second course of chemotherapy without Rikkunshito. Treatment with reversed order was performed for Group B (n = 5). Patients in the intervention period took Rikkunshito (Tsumura Co, Ltd, Tokyo, Japan) at a daily dose of 7.5 g (2.5 g three times daily, orally) before every meal through chemotherapy from days 1 to 21. On the other hand, patients in the control period took nothing. All patients received combined chemotherapy with S-1 plus cisplatin as follows. S-1 (Taiho Pharmaceutical Co, Ltd, Tokyo, Japan) was given orally twice daily after meals at a fixed dose of 80 mg/m^2/day for 21 consecutive days followed by a 14-day rest period; this cycle was repeated every 5 weeks. The dose of S-1,

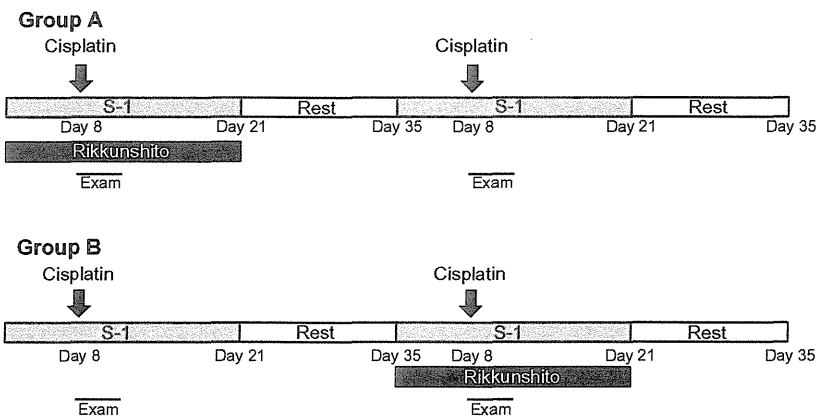

Figure 1 Crossover study design. Patients in group A initially took oral Rikkunshito before every meal for 3 weeks (on treatment). After a rest period of 2 weeks, Rikkunshito was discontinued for 3 weeks (off treatment). Conversely, patients in group B initially were off treatment for 3 weeks and then on treatment for 3 weeks after the rest period.

Table 1 Background of patients

Patient	Age (years)	Sex	Tumor	PS	Group
1	55	M	Recurrent	1	A
2	61	F	Unresectable	0	A
3	67	M	Recurrent	0	B
4	71	M	Unresectable	0	A
5	52	M	Unresectable	1	A
6	72	M	Recurrent	0	B
7	50	F	Unresectable	1	B
8	62	M	Unresectable	0	B
9	67	M	Recurrent	1	A
10	61	M	Unresectable	0	B

Abbreviation: PS, performance status.

decided on the basis of body surface area (BSA), was 80 mg (BSA <1.25 m^2), 100 mg (BSA 1.25–1.5 m^2), or 120 mg (BSA ≥ 1.5 m^2). Cisplatin (Landa™, Nippon Kayaku Co, Ltd, Tokyo, Japan) was administered intravenously on day 8. All patients received 16 mg of dexamethasone and 3 mg of granisetron intravenously one hour before cisplatin infusion and 8 mg of dexamethasone on days 9 and 10. The initially administered dose of cisplatin was 60 mg/m^2. Blood samples were obtained from each patient before the administration of cisplatin on day 8 and 3 hours after the administration of cisplatin was finished. The primary endpoint was the amount of oral intake, and the categories of the scales of anorexia, nausea, and vomiting; secondary endpoints included the plasma concentration of acylated ghrelin.

Measurement of acylated ghrelin

The blood samples were collected before and 3 hours after the administration of cisplatin. The sample collecting time was determined on the basis of our unpublished experimental data from dogs. The plasma samples for acylated ghrelin were promptly centrifuged at 4°C, and the supernatants were acidified with 1 mol/L HCl (1/10 volume), frozen, and kept below 40°C until measurement. The acylated ghrelin level was determined using the active ghrelin enzyme-linked immunosorbent assay kit (SCETI Co, Ltd, Tokyo, Japan).

Definition of response

Following administration of cisplatin, each patient was hospitalized and monitored by direct observation and patient interview for 5 days. The amount of oral intake of each meal was observed and scored by 11 stages from 0 to 10 by nurses, and the average oral intake during 5 days was calculated and analyzed. Categories of the scales for anorexia, nausea, and vomiting were graded according to the National Cancer Institute common toxicity criteria, version 3.0.[15] We defined the time to treatment failure as the period between the time that administration of cisplatin was finished and the time that vomiting or dry vomiting occurred or the time of administration of the antiemetic.

Statistical analysis

The results were expressed as the mean ± the standard error of the mean. The Student's *t*-test was used to test for the significance of differences between groups. For the comparison of time to treatment failure between two groups, the Kaplan-Meier product-limit method and log-rank test were used. A P value <0.05 was considered statistically significant. Statistical calculations were performed using StatView® software (version 5.0, Abacus Concepts Inc, Berkeley, CA).

Results

Plasma concentration of acylated ghrelin

In the Rikkunshito-off period, the average concentration of plasma acylated ghrelin 3 hours after the administration of cisplatin decreased from that before administration of cisplatin, but the difference was not significant. On the other hand, in the Rikkunshito-on period, no decrease in plasma concentration of acylated ghrelin was observed between before and after administration (Figure 2).

Amount of oral intake

The average oral intake in the Rikkunshito-on period was significantly larger than that in the Rikkunshito-off period (6.29 versus 3.94, $P = 0.0496$, Figure 3). This tendency was similarly seen in group A and group B, and neither an order effect nor a carry-over effect was seen.

Anorexia, nausea, vomiting

The grade of anorexia (0–4) was significantly lower in the Rikkunshito-on period than in the Rikkunshito-off period

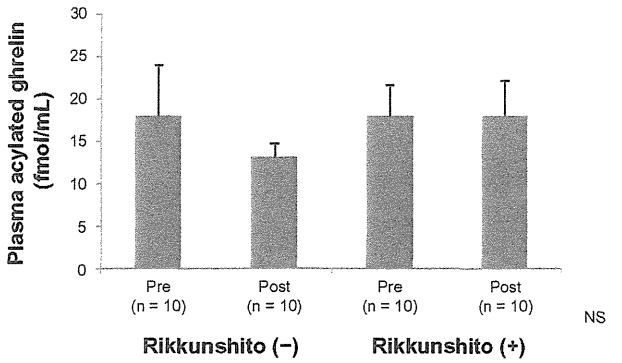

Figure 2 Plasma concentration of acylated ghrelin. In the Rikkunshito-on period (Rikkunshito [+]), no decrease of plasma concentration of acylated ghrelin was observed before and after administration.
Abbreviation: NS, not significant.

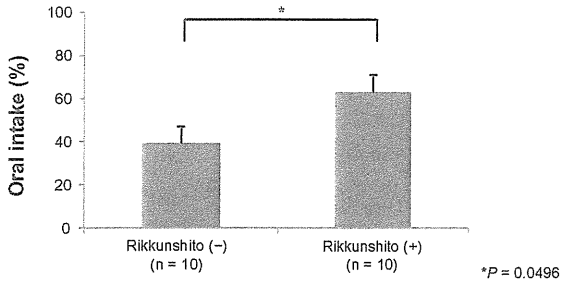

Figure 3 Comparison of the amount of oral intake. The average oral intake in the Rikkunshito-on period was significantly larger than that in the Rikkunshito-off period.
Note: *$P = 0.0496$.

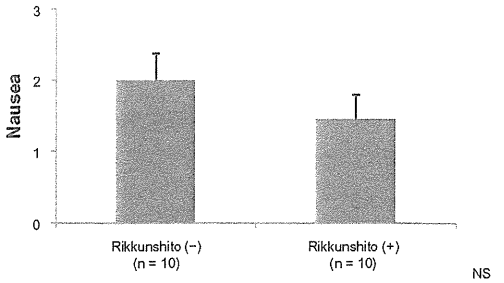

Figure 5 Comparison of the grade of nausea. The difference in the grade of nausea (0–3) was not significant.
Abbreviation: NS, not significant.

(1.2 versus 2.2, $P = 0.0441$, Figure 4). No order effect or carry-over effect was seen. The difference in the grade of nausea (0–3) was not significant, although the grade in the Rikkunshito-on period tended to be lower (Figure 5). The difference in grade of vomiting (0–4) was not significant (Figure 6).

Time to treatment failure

The number of cases of treatment failure in the Rikkunshito-off period was nine, whereas that in the Rikkunshito-on period was five. However, the difference was not significant (Figure 7).

Toxicity

Pseudohyperaldosteronism and hepatic toxicity have been reported as side effects of Rikkunshito. We assessed hypertension, edema, hypokalemia, and transaminitis. These events did not appear for either the Rikkunshito-on or the Rikkunshito-off period, and all patients were able to complete the examination.

Discussion

Ghrelin has been measured in patients with various diseases, for example, functional dyspepsia,[16] breast cancer, colon cancer,[17] and hepatocellular carcinoma,[18] and the relationship between the disease and the level of ghrelin has been assessed. Garcia et al[19] reported that plasma ghrelin levels were higher in cachectic patients than in noncachectic ones, suggesting that an increase in the plasma ghrelin level might be related to secondary loss of appetite in patients. The plasma ghrelin level in patients with obesity was low, and the decrease in body weight was proportional to increasing ghrelin levels.[20] The ghrelin level of patients with anorexia nervosa was reported to be high.[21] It remains controversial whether the plasma ghrelin level of patients with functional dyspepsia increases or decreases.[22,23] Akamizu et al[24] experimented with the administration of ghrelin in patients with functional dyspepsia; their results showed that daily food intake tended to increase in comparison with intake before and after completion of treatment. However, the difference was not significant. The hunger sensation was reported to be significantly elevated. It is certain that ghrelin is related to appetite; however, the manner in which it affects appetite and food intake remains unclear.

Rikkunshito is a traditional Japanese medicine (Kampo) that is widely used for treating upper gastrointestinal symptoms, such as decreased gastric motility after surgery[11] and chronic idiopathic dyspepsia,[12] and its beneficial effects have been shown. Rikkunshito contains mixed raw herbs in the following ratios: *JP Atractylodes lancea rhizome* 4.0 g, *JP Ginseng* 4.0 g, *JP Pinellia tuber* 4.0 g, *JP Poria*

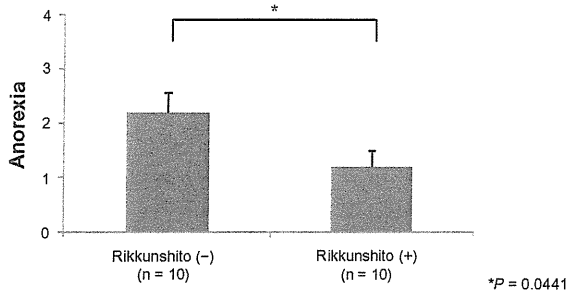

Figure 4 Comparison of the grade of anorexia. The grade of anorexia (0–4) was significantly lower in the Rikkunshito-on period than in the Rikkunshito-off period.
Note: *$P = 0.0441$.

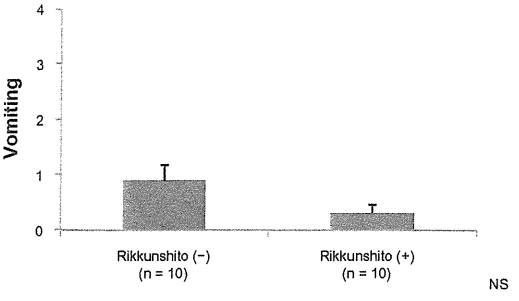

Figure 6 Comparison of the grade of vomiting. The difference in the grade of vomiting (0–4) was not significant.
Abbreviation: NS, not significant.

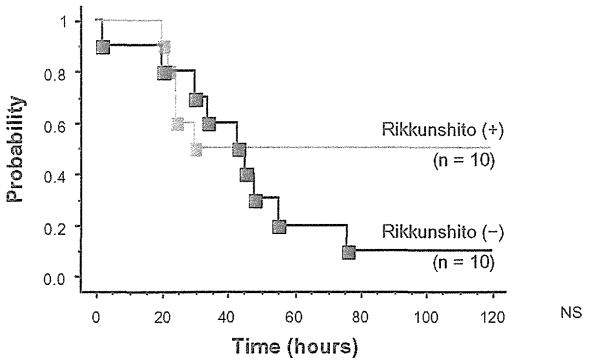

Figure 7 Kaplan–Meier curves of time to treatment failure. The number of cases of treatment failure in the Rikkunshito-off period was nine, whereas that in the Rikkunshito-on period was five.
Abbreviation: NS, not significant.

sclerotium 4.0 g, *JP Jujube* 2.0 g, *JP Citrus unshiu peel* 2.0 g, *JP Glycyrrhiza* 1.0 g, and *JP Ginger* 0.5 g. Rikkunshito has been approved for medicinal use by the Japanese Ministry of Health and Welfare, although the mechanism by which Rikkunshito alleviates upper gastrointestinal symptoms has not been clarified. Recently, Rikkunshito was used in combination with granisetron to alleviate the side effects of anticancer drugs, without affecting their efficacy.[12] Decreased gastric motility and decreased appetite as a result of surgery, anticancer drugs, and functional dyspepsia may be caused by a decreased plasma ghrelin level[25,26] or ghrelin function. In clinical applications, Rikkunshito is particularly effective against functional dyspepsia,[11] and its efficacy is also supported by basic research in rats with a delayed gastric emptying function.[27] Matsumura et al[28] reported that Rikkunshito significantly increased the plasma acylated ghrelin level in healthy volunteers and normal mice, and the ghrelin mRNA expression level in the stomach was upregulated in mice. These findings suggest the possibility that Rikkunshito affects the secretion or function of ghrelin.

Takeda et al[14] reported that Rikkunshito suppresses a cisplatin-induced decrease in the plasma level of ghrelin, a hormone that stimulates gastrointestinal motility and food intake in rats. Heptomethoxyflavone, hesperidin, and isoliquiritigenin, the flavonoids in Rikkunshito, are reported to be responsible for a $5HT_{2B}$ antagonistic effect and restoration of the plasma level of ghrelin.

In the present study, in the Rikkunshito-off period, the average concentration of plasma acylated ghrelin after administration decreased from that before administration, but the difference was not significant. On the other hand, in the Rikkunshito-on period, a decrease of plasma concentration of acylated ghrelin was not observed before nor after administration (Figure 2). This result does not contradict the findings of Takeda et al. Rikkunshito might restore the plasma level of ghrelin.

The average oral intake in the Rikkunshito-on period was significantly larger than that in the Rikkunshito-off period (Figure 3), and the grade of anorexia was significantly lower in the Rikkunshito-on period than that in the Rikkunshito-off period (Figure 4). These results might also indicate the effect of restoration of ghrelin. On the other hand, the differences in the grade of nausea, vomiting, and time to treatment failure between the two groups were not significant (Figures 5, 6, and 7). Nausea and vomiting are reactions mainly related to $5HT_3$ receptors, and the relationship with ghrelin might be minimal.

In summary, Rikkunshito might prevent anorexia induced by cisplatin; thus, prophylactic administration was effective in chemotherapy with cisplatin, and patients could continue their treatment on schedule. This is the first report demonstrating the beneficial effects of Rikkunshito in the treatment of cisplatin therapy in humans. A limitation of the present study is the small number of patients. It will be necessary to confirm the usefulness of Rikkunshito by performing larger randomized controlled studies in the future.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Koizumi W, Tanabe S, Saigenji K, et al. Phase I/II study of S-1 combined with cisplatin in patients with advanced gastric cancer. *Br J Cancer*. 2003;89(12):2207–2212.
2. Koizumi W, Narahara H, Hara T, et al. S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. *Lancet Oncol*. 2008;9(3):215–221.
3. Longo F, Mansueto G, Lapadula V, et al. Palonosetron plus 3-day aprepitant and dexamethasone to prevent nausea and vomiting in patients receiving highly emetogenic chemotherapy. *Support Care Cancer*. 2011; 19(8):1159–1164.
4. Aapro M, Fabi A, Nole F, et al. Double-blind, randomised, controlled study of the efficacy and tolerability of palonosetron plus dexamethasone for 1 day with or without dexamethasone on days 2 and 3 in the prevention of nausea and vomiting induced by moderately emetogenic chemotherapy. *Ann Oncol*. 2010;21(5):1083–1088.
5. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*. 1999;402(6762):656–660.
6. Nakazato M, Murakami N, Date Y, et al. A role for ghrelin in the central regulation of feeding. *Nature*. 2001;409(6817):194–198.
7. Wren AM, Seal LJ, Cohen MA, et al. Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab*. 2001;86(12): 5992.
8. Masuda Y, Tanaka T, Inomata N, et al. Ghrelin stimulates gastric acid secretion and motility in rats. *Biochem Biophys Res Commun*. 2000; 276(3):905–908.
9. Asakawa A, Inui A, Kaga T, et al. Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. *Gut*. 2003;52(7):947–952.

10. Hotta M, Ohwada R, Akamizu T, Shibasaki T, Takano K, Kangawa K. Ghrelin increases hunger and food intake in patients with restricting-type anorexia nervosa: a pilot study. *Endocr J.* 2009;56(9):1119–1128.
11. Tatsuta M, Iishi H. Effect of treatment with liu-jun-zi-tang (TJ-43) on gastric emptying and gastrointestinal symptoms in dyspeptic patients. *Aliment Pharmacol Ther.* 1993;7(4):459–462.
12. Tomono H, Ito Y, Watanabe T. Successful antiemetic treatment of TSUMURA Rikkunshi-to Extract Granules for ethical use in addition to other antiemetic agents in neoadjuvant chemotherapy for an advanced breast cancer patient. *Gan To Kagaku Ryoho.* 2006;33(8):1129–1131. Japanese.
13. Oka T, Tamagawa Y, Hayashida S, Kaneda Y, Kodama N, Tsuji S. Rikkunshi-to attenuates adverse gastrointestinal symptoms induced by fluvoxamine. *Biopsychosoc Med.* 2007;1:21.
14. Takeda H, Sadakane C, Hattori T, et al. Rikkunshito, an herbal medicine, suppresses cisplatin-induced anorexia in rats via 5-HT_2 receptor antagonism. *Gastroenterology.* 2008;134(7):2004–2013.
15. Japanese translation of common terminology criteria for adverse events (CTCAE), and instructions and guidelines. *Int J Clin Oncol.* 2004;9 Suppl 3:1–82.
16. Shinomiya T, Fukunaga M, Akamizu T, et al. Plasma acylated ghrelin levels correlate with subjective symptoms of functional dyspepsia in female patients. *Scand J Gastroenterol.* 2005;40(6):648–653.
17. Wolf I, Sadetzki S, Kanety H, et al. Adiponectin, ghrelin, and leptin in cancer cachexia in breast and colon cancer patients. *Cancer.* 2006;106(4):966–973.
18. Ataseven H, Bahcecioglu IH, Kuzu N, et al. The levels of ghrelin, leptin, TNF-alpha, and IL-6 in liver cirrhosis and hepatocellular carcinoma due to HBV and HDV infection. *Mediators Inflamm.* 2006;2006(4):78380.
19. Garcia JM, Garcia-Touza M, Hijazi RA, et al. Active ghrelin levels and active to total ghrelin ratio in cancer-induced cachexia. *J Clin Endocrinol Metab.* 2005;90(5):2920–2926.
20. Soriano-Guillen L, Barrios V, Campos-Barros A, Argente J. Ghrelin levels in obesity and anorexia nervosa: effect of weight reduction or recuperation. *J Pediatr.* 2004;144(1):36–42.
21. Dostalova I, Haluzik M. The role of ghrelin in the regulation of food intake in patients with obesity and anorexia nervosa. *Physiol Res.* 2009;58(2):159–170.
22. Takamori K, Mizuta Y, Takeshima F, et al. Relation among plasma ghrelin level, gastric emptying, and psychologic condition in patients with functional dyspepsia. *J Clin Gastroenterol.* 2007;41(5):477–483.
23. Pilichiewicz AN, Feltrin KL, Horowitz M, et al. Functional dyspepsia is associated with a greater symptomatic response to fat but not carbohydrate, increased fasting and postprandial CCK, and diminished PYY. *Am J Gastroenterol.* 2008;103(10):2613–2623.
24. Akamizu T, Iwakura H, Ariyasu H, et al. Repeated administration of ghrelin to patients with functional dyspepsia: its effects on food intake and appetite. *Eur J Endocrinol.* 2008;158(4):491–498.
25. Shinomiya T, Fukunaga M, Akamizu T, et al. Plasma acylated ghrelin levels correlate with subjective symptoms of functional dyspepsia in female patients. *Scand J Gastroenterol.* 2005;40(6):648–653.
26. Isomoto H, Ueno H, Nishi Y, et al. Circulating ghrelin levels in patients with various upper gastrointestinal diseases. *Dig Dis Sci.* 2005;50(5):833–838.
27. Kido T, Nakai Y, Kase Y, et al. Effects of rikkunshi-to, a traditional Japanese medicine, on the delay of gastric emptying induced by N(G)-nitro-L-arginine. *J Pharmacol Sci.* 2005;98(2):161–167.
28. Matsumura T, Arai M, Yonemitsu Y, et al. The traditional Japanese medicine Rikkunshito increases the plasma level of ghrelin in humans and mice. *J Gastroenterol.* 2010;45(3):300–307.

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Goshajinkigan reduces oxaliplatin-induced peripheral neuropathy without affecting anti-tumour efficacy in rodents

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ABSTRACT

Oxaliplatin is a key drug in the treatment of colorectal cancer, but it causes acute and chronic neuropathies in patients. Goshajinkigan (GJG) is a Kampo medicine that is used for the treatments of several neurological symptoms including pain and numbness. More recently, GJG has been reported to prevent the oxaliplatin-induced peripheral neuropathy in clinical studies. No experimental study, however, has been conducted to date to determine the effect of GJG on pain behaviour in a rat model of oxaliplatin-induced neuropathy. Moreover, the impact on the anti-tumour effect of oxaliplatin remains unknown. In the present study, we examined the effects of GJG on the peripheral neuropathy and anti-tumour activity of oxaliplatin in rodents. Repeated administration of oxaliplatin caused cold hyperalgesia from days 3 to 37 and mechanical allodynia from days 21 to 28. Repeated administration of GJG prevented the oxaliplatin-induced cold hyperalgesia but not mechanical allodynia and axonal degeneration in rat sciatic nerve. Single administration of GJG reduced both cold hyperalgesia and mechanical allodynia after the development of neuropathy. In addition, GJG did not affect the anti-tumour effect of oxaliplatin in the tumour cells or tumour cells-implanted mice. These results suggest that GJG relieves the oxaliplatin-induced cold hyperalgesia and mechanical allodynia without affecting anti-tumour activity of oxaliplatin, and, therefore, may be useful for the oxaliplatin-induced neuropathy in clinical practice.

1. Introduction

Oxaliplatin, a platinum-based chemotherapeutic agent, is widely used for colorectal cancer. However, it causes severe acute and chronic peripheral neuropathies. Acute neuropathy is peculiar to oxaliplatin and includes acral paresthesias enhanced by exposure to cold.[1–4] The acute neuropathy is thought to be not due to morphological damage of the nerve.[5,6] On the other hand, the chronic neuropathy is characterised by sensory and motor neuropathy after long-term treatment with oxaliplatin and it is similar to cisplatin-induced neurological symptom.[3] This chronic neuropathy is often a dose-limiting toxicity.[7,8] For this reason, peripheral neuropathy associated with the administration of oxaliplatin is a major clinical problem in chemotherapy.

The OPTIMOX (stop and go) approach offers a reasonably good strategy[9] and attempts to prevent oxaliplatin-induced neuropathy, but it has not been successful well. Gamelin et al.[10,11] reported that intravenous administration of calcium gluconate and magnesium sulphate (Ca/Mg) before and after oxaliplatin therapy could alleviate peripheral neurotoxicity, but the injections of these drugs make the chemotherapy

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regimen cumbersome and complicated. Therefore, a preventive agent for neuropathy has not yet been established. Oxaliplatin is metabolised to oxalate and dichloro(1,2-diaminocyclohexane) platinum [Pt(dach)Cl_2].[12] We previously demonstrated that repeated administration of oxaliplatin (4 mg/kg) induced cold hyperalgesia in the early phase and mechanical allodynia in the late phase in rats, and that oxalate is involved in the cold hyperalgesia but not mechanical allodynia.[13] Moreover, we indicated that pre-administration of Ca or Mg prevents the cold hyperalgesia but not mechanical allodynia which is related to Pt(dach)Cl_2.[13]

Goshajinkigan (GJG), a Kampo medicine, has widely been used to treat symptoms like numbness, vibration sensation, cold sensation and limb pain associated with diabetic neuropathy.[14–16] More recently, GJG has been shown to prevent the oxaliplatin-induced peripheral neuropathy in clinical studies.[17,18] No experimental study, however, has been conducted to date to determine the effect of GJG on pain behaviour in an animal model of oxaliplatin-induced neuropathy. Moreover, the impact on the anti-tumour effect of oxaliplatin remains unknown. In the present study, we examined the effects of GJG on the peripheral neuropathy and anti-tumour activity of oxaliplatin in rodents.

2. Materials and methods

2.1. Animals

Six-week-old male Sprague–Dawley rats weighing 200–250 g (Kyudo Co., Saga, Japan) were used for the oxaliplatin-induced peripheral neuropathy model. Six-week-old male BALB/c mice weighing 21–23 g (CLEA Japan, Inc., Tokyo, Japan) were used for the *in vivo* tumour growth model. They were housed in groups of four to five per cage, with lights on from 07:00 to 19:00 h. Animals had free access to food and water in their home cages. All experiments were approved by the Experimental Animal Care and Use Committee of Kyushu University according to the National Institutes of Health guidelines, and we followed International Association for the Study of Pain (IASP) Committee for Research and Ethical Issues guidelines for animal research.[19]

2.2. Drugs

Oxaliplatin (Elplat®) was obtained from Yakult Co., Ltd. (Tokyo, Japan) and was dissolved in 5% glucose solution. GJG (Lot. No. 2090107010) was a generous gift from Tsumura & CO. (Tokyo, Japan). In the oxaliplatin-induced peripheral neuropathy model, oxaliplatin (4 mg/kg) or vehicle (5% glucose solution) was injected intraperitoneally (i.p.) twice a week for 4 weeks (days 1, 2, 8, 9, 15, 16, 22 and 23). Oxaliplatin was administered at a volume of 1 mL/kg of body weight. GJG (0.3 and 1.0 g/kg) was dissolved in distilled water. The doses of these drugs were chosen based on a previous report.[13,20–23] Behavioural tests were performed blindly with respect to drug administration.

2.3. Acetone test for cold hyperalgesia

The cold hyperalgesia was assessed by acetone test described by Flatters and Bennett.[24] 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., Osaka, Japan) was sprayed onto the plantar skin of each hind paw three times with a Micro Sprayer® (Penn Century Inc., Philadelphia, PA, Unites States of America), and the number of withdrawal response was counted for 40 s from the start of the acetone spray.

2.4. von Frey test for mechanical allodynia

The mechanical allodynia was assessed by von Frey test. 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 (The Touch Test Sensory Evaluator Set; Linton Instrumentation, Norfolk, United Kingdom) ranging 1–15 g bending force were applied to the midplantar skin of each hind paw with each application held for 6 s. Withdrawal responses to the stimulation of von Frey filaments were monitored and paw withdrawal thresholds were determined by a modified up-down method.[23]

2.5. Experimental schedule

To examine the preventive effect of repeated administration of GJG on the oxaliplatin-induced cold hyperalgesia and mechanical allodynia, GJG was administered p.o. once 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, 30 and 37. On days 1, 3, 5, 7, 14 and 21, this test was performed before drug administration. The von Frey test was performed before the first drug administration (on day 0) and on days 5, 15, 21 and 28. This behavioural test was performed before drug administration.

Next, we examined the therapeutic effect of single administration of GJG on the oxaliplatin-induced cold hyperalgesia and mechanical allodynia after the development of neuropathy. We confirmed the incidence of cold hyperalgesia and mechanical allodynia on day 5 and day 28, respectively. We carried out the drug evaluation the next day. GJG was administered p.o. The acetone test was performed immediately before (0 min) and at 30, 60, 90, 120, 150 and 180 min after administration. The von Frey test was performed immediately before (0 min) and at 30, 60, 90 and 120 min after administration. GJG was administered at a volume of 5 mL/kg of body weight.

2.6. Assay of sciatic nerve axonal degeneration

On day 28, sciatic nerves were harvested from rats anesthetised with sodium pentobarbital. Nerves were fixed in 2% (w/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.4, 4 °C) for 4 h followed by wash with 0.1 M phosphate buffer. After 8% (w/v) sucrose-substitution, samples were embedded in Epon. Each section was stained with toluidine blue. Sample sections were evaluated using light microscopy (BX51; Olympus Corp., Tokyo, Japan).

2.7. Cell cultures

Murine colon adenocarcinoma 26 (C-26) cells were obtained from the Riken (Saitama, Japan). C-26 cells were maintained

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in RPMI 1640 medium (MP Biomedicals Inc., Irvine, CA, USA) containing 2 mM L-glutamine, 10% foetal bovine serum in a humidified atmosphere containing 5% CO_2 at 37 °C.

2.8. *Tumour cytotoxicity assay*

C-26 cells were seeded at a density of 2×10^4 cells/cm^2 onto 24 well plates and were used for experiments on the following day. Cells were exposed to oxaliplatin (10 ng/mL) and GJG (10, 30, 100,or 300 μg/mL) for 12, 24 or 48 h. Oxaliplatin and GJG were dissolved in medium. The cell viability was assessed by the mitochondrial activity in reducing WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2 H-tetrazolium, monosodium salt) to formazan. At 12, 24, or 48 h after incubation with oxaliplatin and GJG, the cells were washed with phosphate-buffered saline, then 210 μL of serum-free medium and 10 μL of WST-8 assay solution (Cell Counting Kit-8; Dojindo Laboratory, Kumamoto, Japan) were added and incubated for 1 h at 37 °C in humidified air supplemented with 5% CO_2. The incubation medium was carefully taken and transferred to 96 well flat-bottom plastic plates (Corning Costar, Corning, NY, USA). The amount of formed formazan dye was measured from the absorbance at 450 nm with a reference wavelength of 620 nm using a microplate reader (Immuno-mini NJ-2300; Inter Medical, Tokyo, Japan).

2.9. *Tumour growth analysis using mouse model*

C-26 cells (1.0×10^6 cells per mouse in 10 μL serum free medium) were implanted subcutaneously in the left paw of

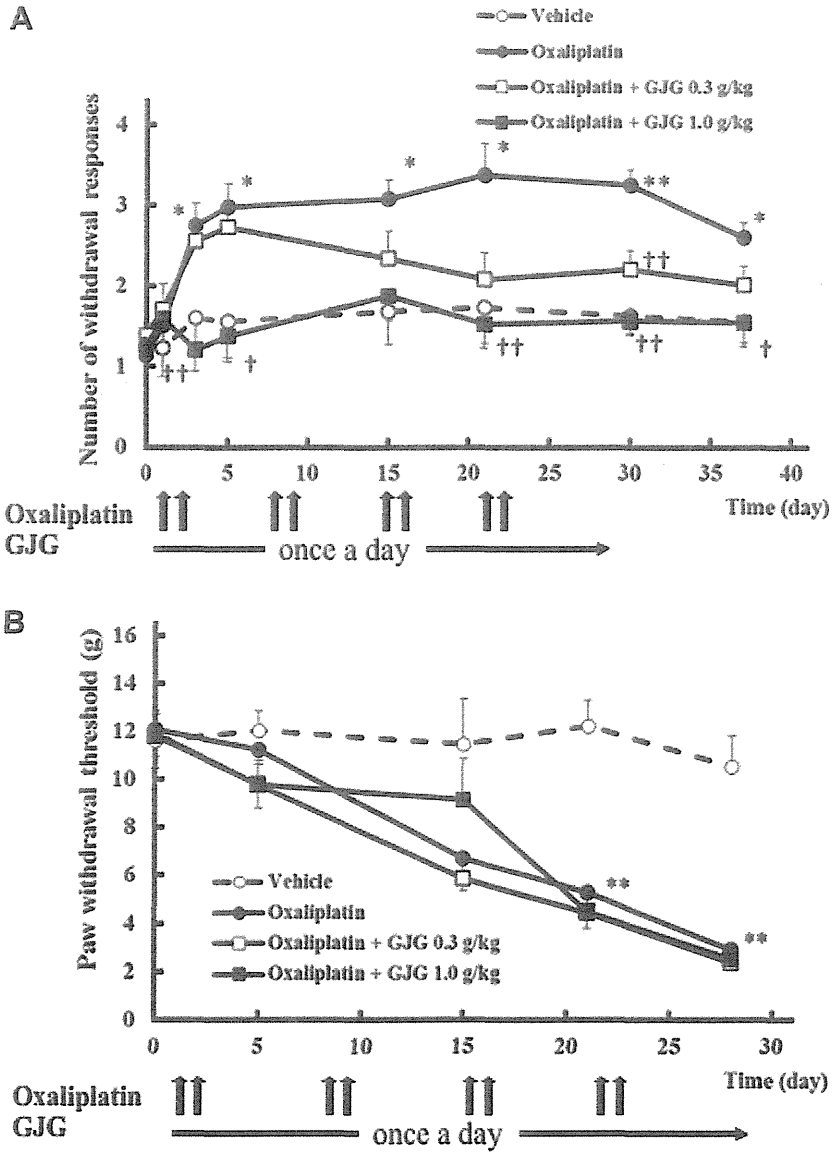

Fig. 1 – Effects of repeated administration of goshajinkigan (GJG) on oxaliplatin-induced cold hyperalgesia and mechanical allodynia in acetone (A) and von Frey (B) tests in rats. Oxaliplatin (4 mg/kg) was administered i.p. twice a week for 4 weeks. GJG (0.3 and 1.0 g/kg) was administered p.o. once a 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, 30 and 37. The von Frey test was performed before the first drug administration (on day 0) and on days 5, 15, 21 and 28. Values are expressed as the mean ± standard error mean of 7–8 animals. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with vehicle. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ compared with oxaliplatin alone.

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BALB/c mice. Three days after implantation of tumour cells, administration of drugs was started. Oxaliplatin (6 mg/kg, i.p.) was injected twice a week and GJG (1.5 g/kg, p.o.) was injected once a day. The tumour volumes were calculated as follows: Volume (mm^3) = 1/2 × Thickness (mm) × Length (mm) × Width (mm).

2.10. *Statistical analyses*

Values were expressed as the mean ± standard error mean. The values were analysed by the Student's t-test, or one-way analysis of variance (ANOVA) followed by the Tukey–Kramer test (StatView; Abacus Concepts, Berkely, CA, USA) to determine differences among the groups. The values of tumour cytotoxicity were expressed as percentages of level of vehicle-treated group. A probability level of $P < 0.05$ was accepted as statistically significant.

3. Result

3.1. *Effect of repeated administration of GJG on cold hyperalgesia in acetone test in oxaliplatin-treated rats*

Oxaliplatin (4 mg/kg, i.p., twice a week) significantly increased the number of withdrawal responses compared with vehicle on days 3, 5, 15, 21, 30 and 37 ($P < 0.05$ or 0.01 by Tukey–Kramer test, Fig. 1A). The repeated administration of GJG (0.3 g/kg, p.o.) weakly reduced the increase of number of withdrawal responses by oxaliplatin (day 30: $P < 0.01$ by Tukey–Kramer test). Moreover, GJG (1.0 g/kg, p.o.) completely reversed the oxaliplatin-induced increase of number of withdrawal responses (days 5 and 37: $P < 0.05$, days 3, 21 and 30: $P < 0.01$ by Tukey–Kramer test).

3.2. *Effect of repeated administration of GJG on mechanical allodynia in von Frey test in oxaliplatin-treated rat*

Oxaliplatin (4 mg/kg, i.p., twice a week) significantly reduced the withdrawal threshold compared with vehicle on days 21 and 28 ($P < 0.01$ by Tukey–Kramer test, Fig. 1B). The repeated administration of GJG (0.3 and 1.0 g/kg) had no effect on the oxaliplatin-induced reduction of withdrawal threshold.

3.3. *Effect of repeated administration of GJG on oxaliplatin-induced histological change in rat sciatic nerve*

No histological abnormalities in sciatic nerve were observed in vehicle-treated rats (Fig. 2). Oxaliplatin (4 mg/kg, i.p., twice a week) induced the decrease in the density of myelinated fibres and the degeneration of myelinated fibres in rat sciatic nerve. These histological changes were also observed in the tissue of rat treated with co-administration of oxaliplatin and GJG.

3.4. *Effect of single administration of GJG on cold hyperalgesia after the development of neuropathy in acetone test in oxaliplatin-treated rats*

Oxaliplatin (4 mg/kg, i.p., twice on days 1 and 2) significantly increased the number of withdrawal responses compared with vehicle in acetone test on day 5 ($P < 0.05$ by Tukey–Kramer test, Fig. 3A). The single administration of GJG (0.3 g/kg) had no effect on the oxaliplatin-induced increase of number of withdrawal responses, while GJG (1.0 g/kg) significantly reduced this response (30 and 90 min: $P < 0.05$, 60 min: $P < 0.01$ by Tukey–Kramer test). This effect of GJG disappeared by 180 min after administration.

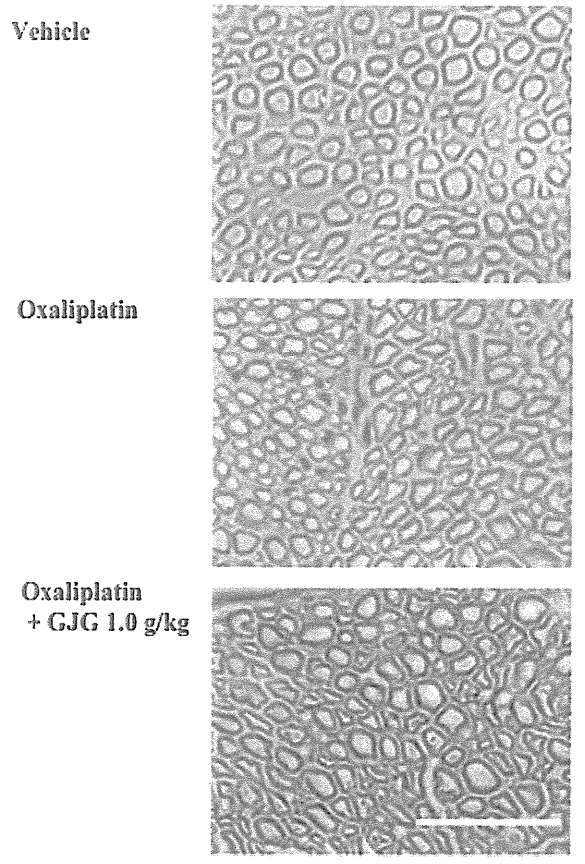

Fig. 2 – Effect of repeated administration of goshajinkigan (GJG) on histological change induced by oxaliplatin in rat sciatic nerve. Rats were treated with oxaliplatin (4 mg/kg, i.p.) twice a week for 4 weeks. GJG (1.0 g/kg) was administered p.o. once a day for 4 weeks. On day 28, the sciatic nerve was harvested, and samples were stained with toluidine blue. Photographs were originally magnified 800×. Scale bar 50 μm.

3.5. *Effect of single administration of GJG on mechanical allodynia after the development of neuropathy in von Frey test in oxaliplatin-treated rats*

Oxaliplatin (4 mg/kg, i.p., twice on days 1, 2, 8, 9, 15, 16, 22 and 23) significantly reduced the withdrawal threshold compared with vehicle on day 28 ($P < 0.01$ by Tukey–Kramer test, Fig. 3B). The single administration of GJG (0.3 g/kg) significantly increased the reduced threshold by oxaliplatin at 30 and 90 min after administration ($P < 0.05$ by Tukey–Kramer test). Similarly, GJG (1.0 g/kg) significantly increased the oxaliplatin-induced reduction of withdrawal threshold at 30 min after administration ($P < 0.01$ by Tukey–Kramer test). These effects of GJG disappeared by 120 min after administration.

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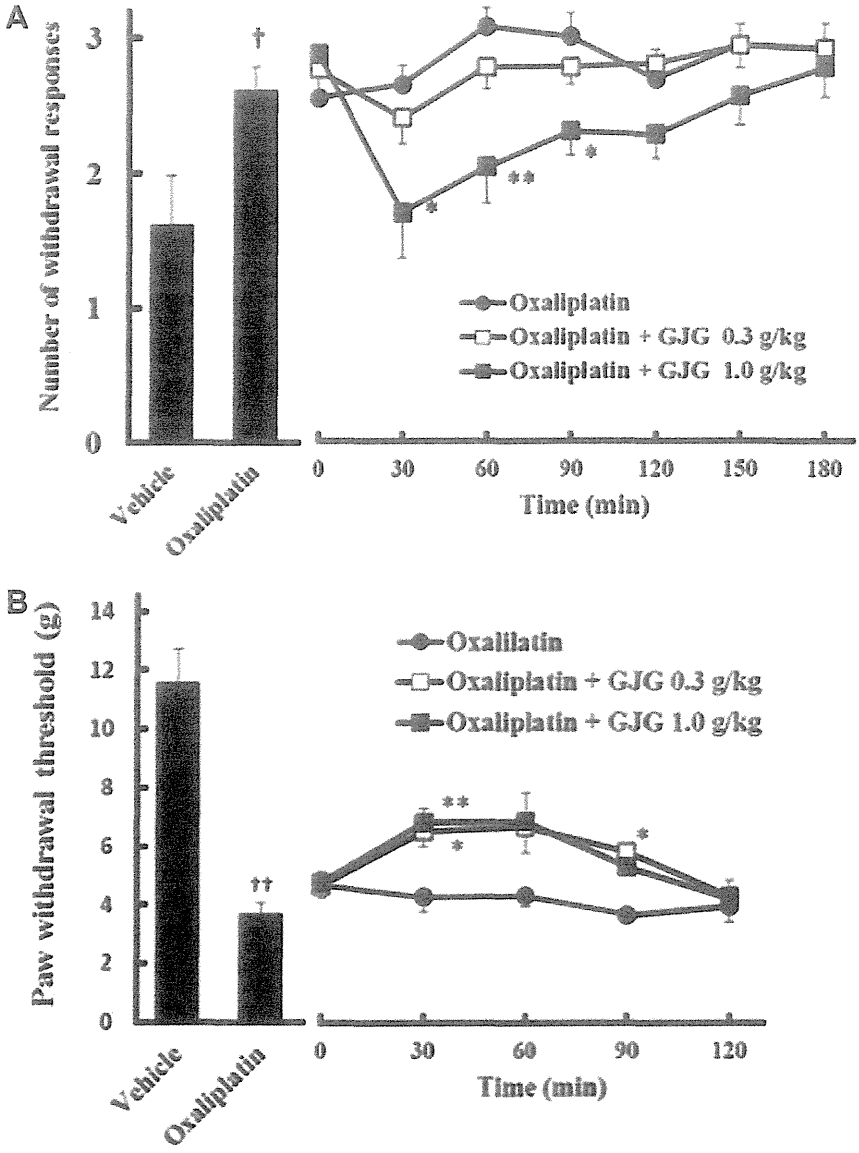

Fig. 3 – Effects of single administration of goshajinkigan (GJG) on the cold hyperalgesia and mechanical allodynia after the development of neuropathy in acetone (A) and von Frey (B) tests in oxaliplatin-treated rats. Rats were treated with oxaliplatin (4 mg/kg, i.p.) twice on days 1 and 2 (A) or twice on days 1, 2, 8, 9, 15, 16, 22 and 23 (B). We confirmed the incidence of cold hyperalgesia and mechanical allodynia on days 5 and 28, respectively. We carried out the drug evaluation the next day. GJG (0.3 and 1.0 g/kg) was administered p.o. Values are expressed as the mean ± standard error mean of 6–8 animals. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ compared with vehicle. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with oxaliplatin alone.

3.6. *Effect of GJG on the tumour cytotoxicity of oxaliplatin*

The exposure of cultured C-26 cells to oxaliplatin (3 μM) for 12, 24 or 48 h caused time-dependent decreases in tumour cell viability as assessed by mitochondrial enzyme activity using the WST-8 assay (Fig. 4). GJG (10–300 μg/mL) had no effect on the oxaliplatin-induced decrease of tumour cell viability in cell line.

3.7. *Effect of GJG on the anti-tumour activity of oxaliplatin in tumour cells-implanted mice*

Oxaliplatin (6 mg/kg, i.p.) significantly inhibited the increase of tumour volumes compared with vehicle on days 11 and 16 in tumour cells-implanted mice ($P < 0.01$ by Tukey–Kramer test, Fig. 5). GJG (1.5 g/kg, p.o.) had no effect on the oxaliplatin-induced inhibition of tumour growth.

4. Discussion

In the present study, oxaliplatin caused cold hyperalgesia from the early phase and mechanical allodynia in the late phase, consistently with our previous reports.[13,23] The repeated administration of GJG reduced the oxaliplatin-induced cold hyperalgesia in the acetone test, whereas it had no effect on the oxaliplatin-induced mechanical allodynia in the von Frey test. Recently, an increased expression of transient receptor potential melastatin 8 (TRPM8) has been

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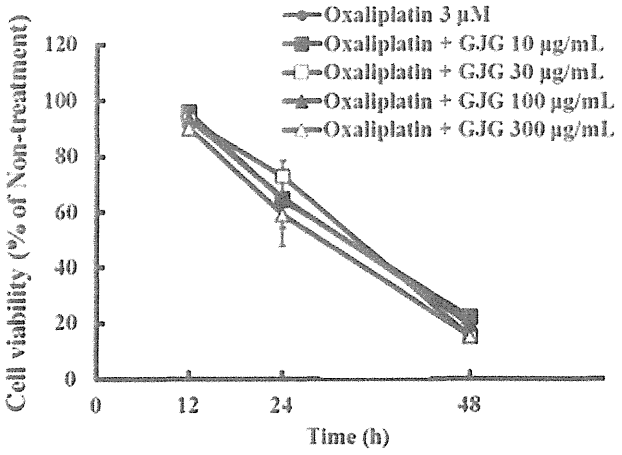

Fig. 4 – Effect of goshajinkigan (GJG) on the tumour cytotoxicity of oxaliplatin. C-26 cells were incubated with oxaliplatin (3 μM) for 12, 24, or 48 h in the presence or absence of various concentrations (10–300 μg/mL) of GJG. Cell viability was measured by WST-8 assay. Values are expressed as percentages of the viability of the vehicle-treated group (n = 6–9).

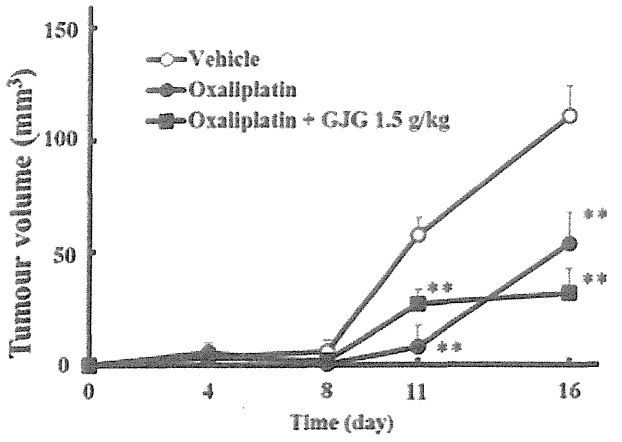

Fig. 5 – Effect of goshajinkigan (GJG) on the anti-tumour effect of oxaliplatin. C-26 cells-implanted mice were treated with oxaliplatin (6 mg/kg, i.p.) twice a week and GJG (1.5 g/kg, p.o.) once a day for 16 days. Values are expressed as the mean ± standard error mean of 12 animals on days 0, 4, 8, 11 and 16. **P < 0.01 compared with vehicle.

reported to be involved in oxaliplatin-induced cold allodynia in mice.[25] Single administration of oxaliplatin increases the expression level of TRPM8 mRNA at day 3 after injection and the expression is decreased to the normal level on day 10. The TRPM8 is activated by cooling temperature, and its mRNA is expressed in dorsal root ganglion, but not in other tissues.[26] Therefore, GJG might prevent the oxaliplatin-induced cold hyperalgesia by inhibiting the expression of TRPM8. We also observed that oxaliplatin caused the degeneration and the decrease in the density of myelinated fibres in rat sciatic nerve on day 28. However, repeated administration of GJG had no effect on the histological changes induced by oxaliplatin. These results suggest that GJG cannot protect against the oxaliplatin-induced axonal degeneration. Recently, we have reported that no histological abnormalities in sciatic nerve were observed in oxaliplatin-treated rats on day 5, although oxaliplatin caused cold hyperalgesia in the acetone test on that day.[23] Therefore, it is unlikely that repeated administration of GJG prevented the oxaliplatin-induced cold hyperalgesia by protecting against the axonal degeneration. In addition, the present results support the involvement of axonal degeneration in the incidence of mechanical allodynia in the late phase but not cold hyperalgesia from the early phase.

Our data in this study revealed that single administration of GJG after the development of neuropathy reduced both cold hyperalgesia and mechanical allodynia. The present results suggest that GJG is useful as symptomatic therapy for oxaliplatin-induced peripheral neuropathy. GJG has been reported to show anti-nociceptive effect based on not only stimulation of spinal κ-opioid receptors via dynorphin release but also increase of peripheral blood flow via increase in nitric oxide production, in streptozotocin-induced diabetic mice.[20,21,27] The herbal medicine component of GJG also has antioxidant properties.[28,29] Furthermore, GJG partially reverses C fibre activation through the reduction of the tachykinins, transient receptor potential vanilloid type 1 (TRPV1) channels and P2X3 purine receptors.[30] Therefore, the effects of GJG on the oxaliplatin-induced cold hyperalgesia and mechanical allodynia might be due to increase of peripheral blood flow, stimulate spinal κ-opioid receptors, inhibit oxidative stress or suppress the C fibre activation. In fact, it has been reported that oxaliplatin gradually decreases peripheral blood flow in mice[31] and increases responses of C-fibre nociceptors to mechanical stimulation in rats.[32] Moreover, both systemic and local administration of antioxidants (acetyl-L-carnitine, alpha-lipoic acid or vitamin C) markedly inhibit the oxaliplatin-induced neuropathy.[32]

In this study, repeated administration of GJG (0.3 g/kg) reduced the cold hyperalgesia in the late phase but not early phase. Though the reason for the effect of GJG is unknown, repeated administration of lower dose of GJG might reduce cold hyperalgesia in the late phase through progressive increase of peripheral blood flow without protecting against the axonal degeneration or inhibiting the expression of TRPM8.

The present results also show that GJG had no effect on the oxaliplatin-induced tumour cytotoxicity in C-26 cells. Furthermore, GJG had no effect on the anti-tumour effect of oxaliplatin in tumour cells-implanted mice. Therefore, it is unlikely that GJG influences the anti-tumour effect of oxaliplatin.

In conclusion, the study presented here demonstrates, for the first time, that GJG ameliorates the oxaliplatin-induced neuropathy in the rat model without affecting the anti-tumour activity of oxaliplatin. However, GJG cannot protect against the oxaliplatin-induced axonal degeneration in rat sciatic nerve. Therefore, GJG is expected to be useful as symptomatic therapy for clinical oxaliplatin-induced neuropathy if it is used with particular care of sensory and motor neuropathies. These data are important information for clinical trials of GJG now underway in particular.

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Conflict of interest statement

None declared.

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REFERENCES

1. Cassidy J, Misset JL. Oxaliplatin-related side effects: characteristics and management. *Semin Oncol* 2002;**29**: 11–20.
2. Extra JM, Marty M, Brienza S, Misset JL. Pharmacokinetics and safety profile of oxaliplatin. *Semin Oncol* 1998;**25**:13–22.
3. Pasetto LM, D'Andrea MR, Rossi E, Monfardini S. Oxaliplatin-related neurotoxicity: how and why? *Crit Rev Oncol Hematol* 2006;**59**:159–68.
4. Quasthoff S, Hartung HP. Chemotherapy-induced peripheral neuropathy. *J Neurol* 2002;**249**:9–17.
5. de Gramont A, Vignoud J, Tournigand C. Oxalipaltin/5FU/LV in adjuvant colon cancer: safety results of the international randomized MOSAIC trial. *Prc Am Soc Clin Oncol* 2002;**21**:132.
6. Wilson RH, Lehky T, Thomas RR, et al. Acute oxaliplatin-induced peripheral nerve hyperexcitability. *J Clin Oncol* 2002;**20**:1767–74.
7. de Gramont A, Figer A, Seymour M, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol* 2000;**18**:2938–47.
8. Misset JL. Oxaliplatin in practice. *Br J Cancer* 1998;**77**:4–7.
9. Tournigand C, Cervantes A, Figer A, et al. OPTIMOX1: a randomized study of FOLFOX4 or FOLFOX7 with oxaliplatin in a stop-and-Go fashion in advanced colorectal cancer – a GERCOR study. *J Clin Oncol* 2006;**24**:394–400.
10. Gamelin E, Gamelin L, Bossi L, Quasthoff S. Clinical aspects and molecular basis of oxaliplatin neurotoxicity: current management and development of preventive measures. *Semin Oncol* 2002;**29**:21–33.
11. Gamelin L, Boisdron-Celle M, Delva R, et al. Prevention of oxaliplatin-related neurotoxicity by calcium and magnesium infusions: a retrospective study of 161 patients receiving oxaliplatin combined with 5-Fluorouracil and leucovorin for advanced colorectal cancer. *Clin Cancer Res* 2004;**10**:4055–61.
12. Graham MA, Lockwood GF, Greenslade D, et al. Clinical pharmacokinetics of oxaliplatin: a critical review. *Clin Cancer Res* 2000;**6**:1205–18.
13. Sakurai M, Egashira N, Kawashiri T, et al. Oxaliplatin-induced neuropathy in the rat: involvement of oxalate in cold hyperalgesia but not mechanical allodynia. *Pain* 2009;**147**:165–74.
14. Tawata M, Kurihara A, Nitta K, et al. The effects of goshajinkigan, a herbal medicine, on subjective symptoms and vibratory threshold in patients with diabetic neuropathy. *Diabetes Res Clin Pract* 1994;**26**:121–8.
15. Uno T, Ohsawa I, Tokudome M, Sato Y. Effects of goshajinkigan on insulin resistance in patients with type 2 diabetes. *Diabetes Res Clin Pract* 2005;**69**:129–35.
16. Usuki Y, Usuki S, Hommura S. Successful treatment of a senile diabetic woman with cataract with goshajinkigan. *Am J Chin Med* 1991;**19**:259–63.
17. Kono T, Mamiya N, Chisato N, et al. Efficacy of goshajinkigan for peripheral neurotoxicity of oxaliplatin in patients with advanced or recurrent colorectal cancer. *Evid Based Complement Alternat Med*, in press.
18. Nishioka M, Shimada M, Kurita N, et al. The Kampo medicine, goshajinkigan, prevents neuropathy in patients treated by FOLFOX regimen. *Int J Clin Oncol*, in press.
19. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 1983;**16**:109–10.
20. Suzuki Y, Goto K, Ishige A, Komatsu Y, Kamei J. Antinociceptive effect of gosha-jinki-gan, a Kampo medicine, in streptozotocin-induced diabetic mice. *Jpn J Pharmacol* 1999;**79**:169–75.
21. Suzuki Y, Goto K, Ishige A, Komatsu Y, Kamei J. Antinociceptive mechanism of Gosha-jinki-gan in streptozotocin-induced diabetic animals: role of nitric oxide in the periphery. *Jpn J Pharmacol* 1999;**79**:387–91.
22. Egashira N, Hirakawa S, Kawashiri T, et al. Mexiletine reverses oxaliplatin-induced neuropathic pain in rats. *J Pharmacol Sci* 2010;**112**:473–6.
23. Kawashiri T, Egashira N, Watanabe H, et al. Prevention of oxaliplatin-induced mechanical allodynia and neurodegeneration by neurotropin in the rat model. *Eur J Pain* 2011;**15**:344–50.
24. Flatters SJ, Bennett GJ. Ethosuximide reverses paclitaxel- and vincristine-induced painful peripheral neuropathy. *Pain* 2004;**109**:150–61.
25. Gauchan P, Andoh T, Kato A, Kuraishi Y. Involvement of increased expression of transient receptor potential melastatin 8 in oxaliplatin-induced cold allodynia in mice. *Neurosci Lett* 2009;**458**:93–5.
26. Peier AM, Moqrich A, Hergarden AC, et al. A TRP channel that senses cold stimuli and menthol. *Cell* 2002;**108**:705–15.
27. Suzuki Y, Goto K, Ishige A, Komatsu Y, Kamei J. Effects of gosha-jinki-gan, a kampo medicine, on peripheral tissue blood flow in streptozotocin-induced diabetic rats. *Method Find Exp Clin Pharmacol* 1998;**20**:321–8.
28. Niwa Y, Miyachi Y. Antioxidant action of natural health products and Chinese herbs. *Inflammation* 1986;**10**:79–91.
29. Kim BJ, Kim JH, Kim HP, Heo MY. Biological screening of 100 plant extracts for cosmetic use (II): anti-oxidative activity and free radical scavenging activity. *Int J Cosmet Sci* 1997;**19**:299–307.
30. Imamura T, Ishizuka O, Aizawa N, et al. Gosha-jinki-gan reduces transmitter proteins and sensory receptors associated with C fibre activation induced by acetic acid in rat urinary bladder. *Neurourol Urodyn* 2008;**27**:832–7.
31. Gauchan P, Andoh T, Kato A, Sasaki A, Kuraishi Y. Effects of the prostaglandin E1 analog limaprost on mechanical allodynia caused by chemotherapeutic agents in mice. *J Pharmacol Sci* 2009;**109**:469–72.
32. Joseph EK, Chen X, Bogen O, Levine JD. Oxaliplatin acts on IB4-positive nociceptors to induce an oxidative stress-dependent acute painful peripheral neuropathy. *J Pain* 2008;**9**:463–72.

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Original Paper

Scheduled Prospective Tri-Weekly Modified FOLFOX6 Maintenance Chemotherapy in the Treatment of Metastatic Colorectal Cancer

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Key Words: Colorectal cancer; Tri-weekly; Maintenance chemotherapy.

ABSTRACT

Background/Aims: Oxaliplatin, which is effective for colorectal cancer (CRC) in combination with 5-fluorouracil (5-FU) and leucovorin (LV), is widely used for metastatic CRC. With the increasing use of oxaliplatin, however, serious adverse events have been experienced, including hematologic and neurologic toxicities. The aim of this study was to evaluate whether tri-weekly modified FOLFOX6 (mFOLFOX6) maintenance chemotherapy is associated with a low incidence of severe hematologic and neurologic toxicities in the treatment of patients with metastatic CRC. **Methodology:** We developed a new treatment regimen with mFOLFOX6 biweekly for 8-10 consecutive cycles (induction phase) followed by a 3-week rest period, after which treatment was resumed with cycles of tri-weekly mFOLFOX6 at standard doses (maintenance phase). Validity and complications were investigated retrospectively. **Results:** Twenty-nine patients were enrolled in this study. The median progression-free survival (PFS) and overall survival (OS) times were 9.4 months and 23 months, respectively. All patients had peripheral neuropathy during treatment, but grade 3 neurotoxicity was observed in only 2 patients (6.9%). **Conclusions:** mFOLFOX6 maintenance chemotherapy was associated with a very low incidence of grade 3 hematologic and neurologic toxicities. The toxicities associated with PFS and OS were comparable to those reported in the treatment of patients with metastatic CRC. A tri-weekly mFOLFOX maintenance strategy of alternative treatment with a less-toxic regimen may reduce toxicity and maintain efficacy.

INTRODUCTION

Colorectal cancer (CRC) is the second-most frequent cause of cancer-related deaths in the United States, with an estimated 150,000 new cases and approximately 50,000 deaths annually (1). Approximately 30% of all patients with CRC have metastatic disease at diagnosis, and 50% of patients with CRC will eventually develop metastatic disease (2). Most patients with metastatic disease are candidates for systemic chemotherapy to palliate symptoms and prolong life. Significant progress in the treatment of CRC has been achieved with the approval of new drugs. An oxaliplatin-containing regimen, FOLFOX, is the current first-line standard therapy for advanced and recurrent CRC (3). The limiting toxicity of FOLFOX is the specific reversible sensory neuropathy of oxaliplatin. The oxaliplatin-related neurotoxicity of the extremities persists between cycles and increases in intensity with the cumulative dose. The cumulative neurotoxicity of oxaliplatin often requires that therapy be stopped in patients who are still responding. Oxaliplatin-induced cumulative neurotoxicity develops progressively in 10-15% of patients after a cumulative dose of 780-800mg/m^2, and severe neuropathy has been reported in the range of 17-21% (3,4). To prevent and manage the development of severe neurotoxicity, intermittent chemotherapy combined with the discontinuation of oxaliplatin treatment in responding patients might be a promising regimen (5). A high rate of neurotoxicity is observed with the use of the FOLFOX regimen at the conventional biweekly schedule. We developed a new regimen with mFOLFOX6 biweekly for 8-10 consecutive cycles (induction phase) followed by a 3-week rest period, after which treatment was resumed with cycles of tri-weekly mFOLFOX6 at standard doses (maintenance phase). The aim of this study was to evaluate whether tri-weekly FOLFOX maintenance chemotherapy is associated with a low incidence of severe neurotoxicity and a better quality of life in patients being treated for metastatic CRC.

METHODOLOGY

Patients with histologically proven CRC who had not previously received chemotherapy for metastatic disease were enrolled in the study. From May 2005 to the present, patients who were given modified FOLFOX6 regimens for the treatment of metastatic CRC were analyzed. A port system (Groshong catheter, MRI port, Bard, Salt Lake City, UT, USA) was implanted in the subclavian vein. Chemotherapy was performed in the out-

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