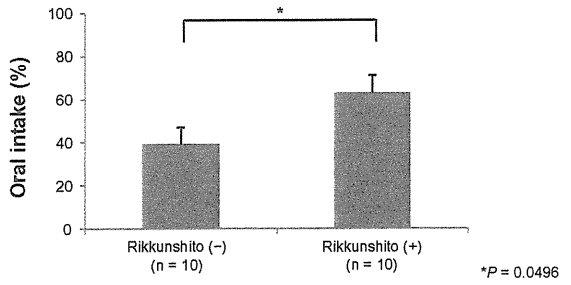

Figure 3 Comparison of the amount of oral intake. The average oral intake in the Rikkunshito-on period was significantly larger than that in the Rikkunshito-off period.
Note: *P = 0.0496.

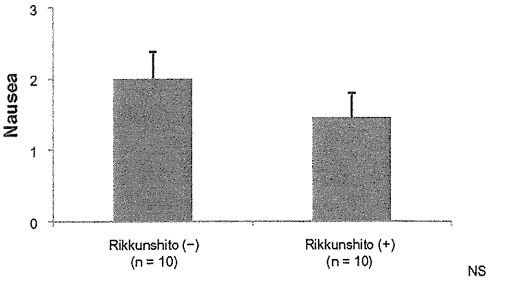

Figure 5 Comparison of the grade of nausea. The difference in the grade of nausea (0–3) was not significant.
Abbreviation: NS, not significant.

(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*

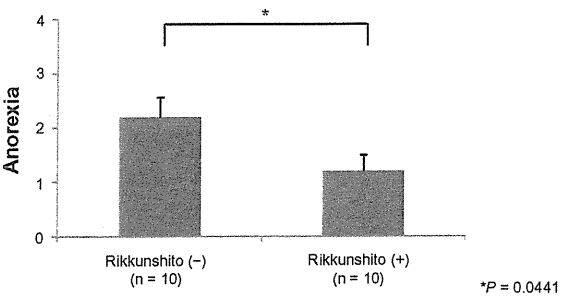

Figure 4 Comparison of the grade of anorexia. The grade of anorexia (0–4) was significantly lower in the Rikkunshito-on period than in the Rikkunshito-off period.
Note: *P = 0.0441.

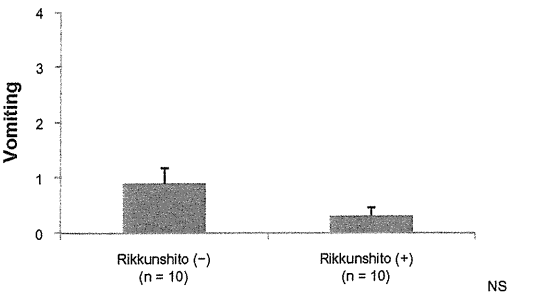

Figure 6 Comparison of the grade of vomiting. The difference in the grade of vomiting (0–4) was not significant.
Abbreviation: NS, not significant.

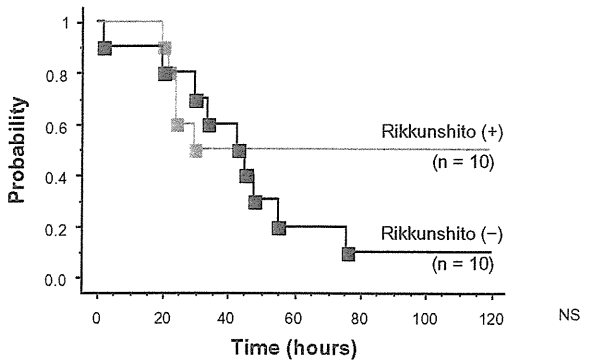

Figure 7 Kaplan–Meier curves of time to treatment failure. The number of cases of treatment failure in the Rikkunshito-off period was nine, whereas that in the Rikkunshito-on period was five.
Abbreviation: NS, not significant.

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.

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

*Soichiro Ushio, Nobuaki Egashira *, Hikaru Sada, Takehiro Kawashiri, Masafumi Shirahama, Ken Masuguchi, Ryozo Oishi*

Department of Pharmacy, Kyushu University Hospital, Fukuoka 812-8582, Japan

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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

* *Corresponding author:* Address: Nobuaki Egashira, Department of Pharmacy, Kyushu University Hospital, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Tel.: +81 92 642 5920; fax: +81 92 642 5937.
E-mail address: n-egashi@pharm.med.kyushu-u.ac.jp (N. Egashira).
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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

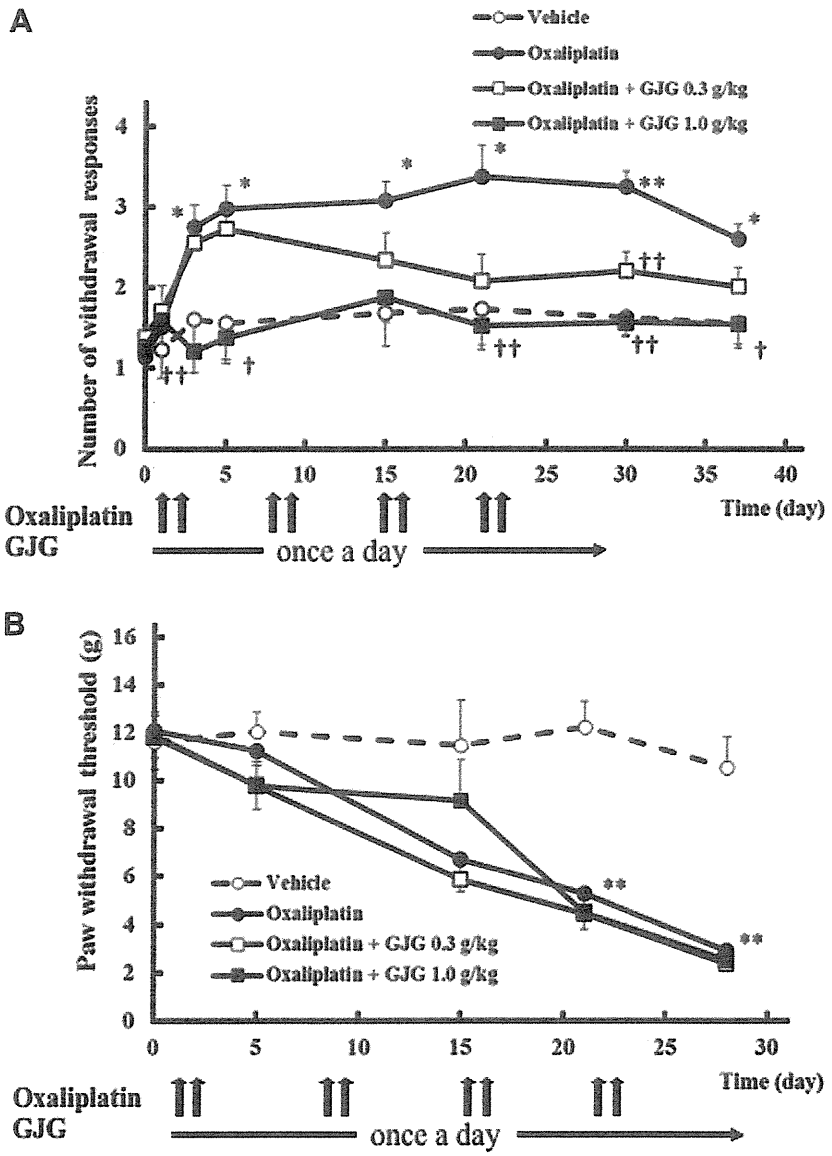

Fig. 1 – Effects of repeated administration of goshajinkigan (GJG) on oxaliplatin-induced cold hyperalgesia and mechanical allodynia in acetone (A) and von Frey (B) tests in rats. Oxaliplatin (4 mg/kg) was administered i.p. twice a week for 4 weeks. GJG (0.3 and 1.0 g/kg) was administered p.o. once a 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. †P < 0.05, ††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.

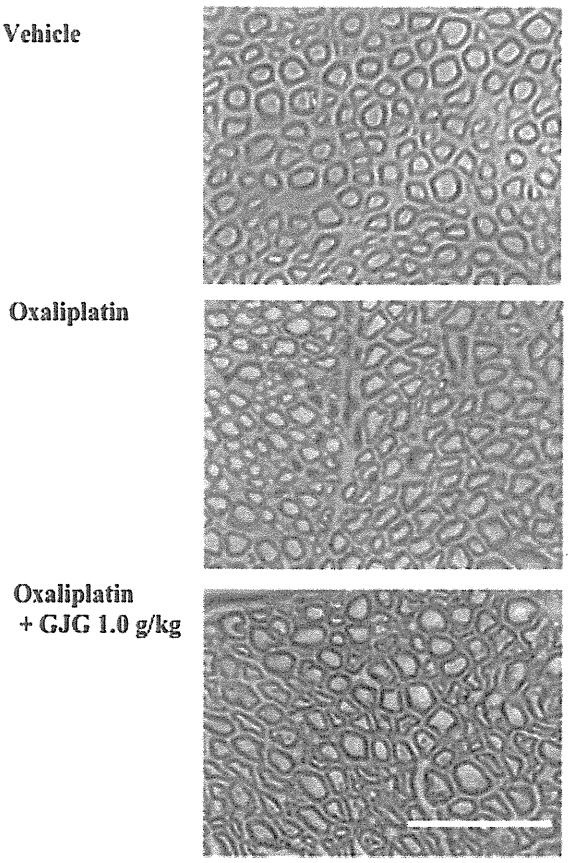

Fig. 2 – Effect of repeated administration of goshajinkigan (GJG) on histological change induced by oxaliplatin in rat sciatic nerve. Rats were treated with oxaliplatin (4 mg/kg, i.p.) twice a week for 4 weeks. GJG (1.0 g/kg) was administered p.o. once a day for 4 weeks. On day 28, the sciatic nerve 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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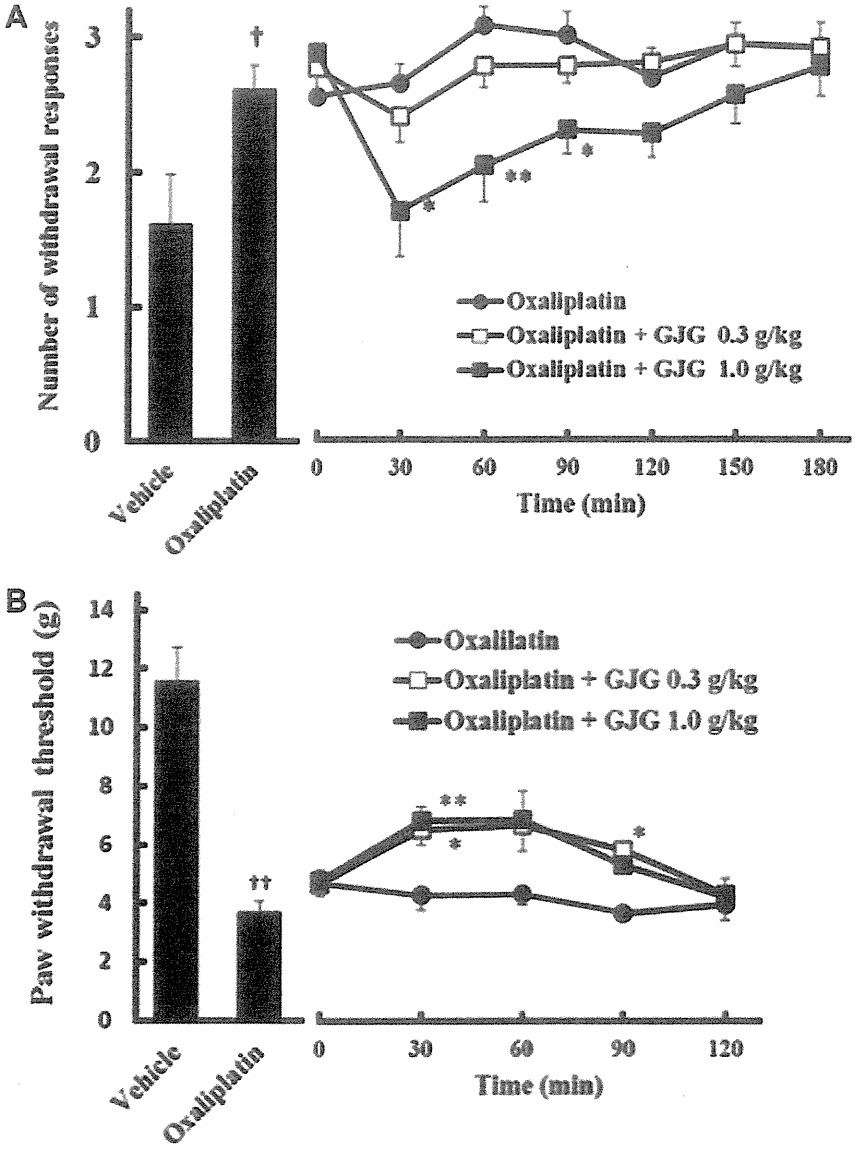

Fig. 3 – Effects of single administration of goshajinkigan (GJG) on the cold hyperalgesia and mechanical allodynia after the development of neuropathy in acetone (A) and von Frey (B) tests in oxaliplatin-treated rats. Rats were treated with oxaliplatin (4 mg/kg, i.p.) twice on days 1 and 2 (A) or twice 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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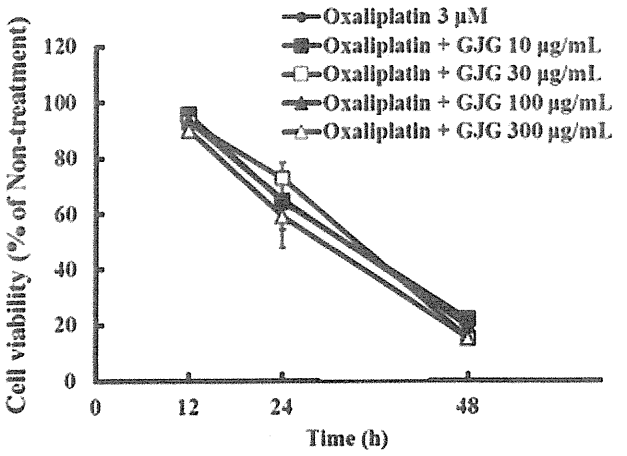

Fig. 4 – Effect of goshajinkigan (GJG) on the tumour cytotoxicity of oxaliplatin. C-26 cells were incubated with oxaliplatin (3 μM) for 12, 24, or 48 h in the presence or absence of various concentrations (10–300 μg/mL) of GJG. Cell viability was measured by WST-8 assay. Values are expressed as percentages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

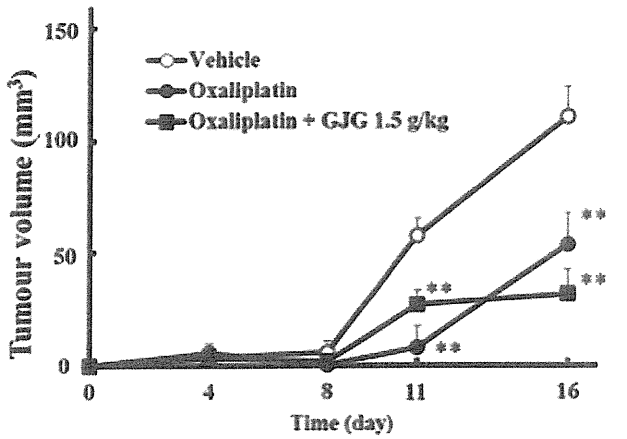

Fig. 5 – Effect of goshajinkigan (GJG) on the anti-tumour effect of oxaliplatin. C-26 cells-implanted mice were treated with oxaliplatin (6 mg/kg, i.p.) twice a week and GJG (1.5 g/kg, p.o.) once a day for 16 days. Values are expressed as the mean ± standard error mean of 12 animals on days 0, 4, 8,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

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

Soichi Tsutsumi, Reina Watanabe, Yuichi Tabe, Takaaki Fujii, Hiroki Morita, Wakako Kigure, Toshihide Kato, Hayato Yamauchi, Takayuki Asao and *Hiroyuki Kuwano*

Department of General Surgical Science (Surgery I), Colorectal Surgical Unit, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan

Corresponding author: Soichi Tsutsumi, M.D., Department of General Surgical Science (Surgery I), Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma, 371-8511, Japan; Tel.: +81-27-220-8224, Fax: +81-27-220-8230; E-mail: chuchumi@showa.gunma-u.ac.jp

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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patient chemotherapy room using the port system and a portable pump.

Regimens

The modified FOLFOX6 regimen included leucovorin 400mg/m^2 intravenous (IV) on day 1, FU 400mg/m^2 IV on day 1 followed by 2,400mg/m^2 IV over 46 hours, and oxaliplatin 85mg/m^2 IV on day 1. All therapy was administered every 2 weeks for a total of 10-13 doses. Treatment was administered until progression of disease or unmanageable toxic effects occurred. After the eighth to tenth FOLFOX cycle, tri-weekly mFOLFOX6 maintenance therapy was initiated. In the case of disease progression, the FOLFIRI (5) regimen could be introduced.

Adverse event reporting

Adverse events were reported using the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. Routine reporting was required for all events ≥ grade 3, every 6 weeks during active treatment.

Dose modifications

Oxaliplatin and FU were reduced for grade 3 and 4 adverse events except for diarrhea and stomatitis, wherein only FU was reduced for less than grade 4 toxicity, or for neurologic toxicities, wherein only oxaliplatin was modified for grades 3 and 2, provided the toxicity exceeded 7 days in duration.

RESULTS

Patient characteristics

Twenty-nine patients with metastatic CRC were treated by the modified FOLFOX6 regimen from May 2005 to December 2009. The patient characteristics are shown in **Table 1.** The patient ages ranged from 39 to 77 years (median 62 years). Nineteen (65.5%) had liver metastases, and 11 (37.9%) had multiple metastatic sites. A total of 446 cycles of treatment were administered with a median of 16 cycles per patient (range 13 to 23 cycles). The median number of tri-weekly mFOLFOX6 cycles administered was 7 (range 5-14). The median cumulative dose of oxaliplatin administered was 1,360mg/m^2 (range 1,105-1,955mg/m^2). After the study, all patients received the FOLFIRI regimen as the second-line chemotherapy in most cases. At a median follow-up of 23 months (**Table 2**), 15 patients were deceased. The median progression-free survival time was 9.4 months.

Toxicity

All patients had peripheral neuropathy during treatment, but grade 3 neurotoxicity was observed in only 2 patients (6.9%). The frequencies of hematologic and non-hematologic toxicity were very low.

DISCUSSION

The current standard first-line chemotherapy for metastatic CRC is the FOLFOX regimen (3,4). Cumulative sensory neurotoxicity is a well-known dose-limiting factor of oxaliplatin (3). Alternative strategies of oxaliplatin-based therapy with decreased cumulative neurologic toxicity, such as the OPTIMOX regimen, have also been examined (6). A high rate of hematological or nonhematological toxicity is observed with the use of the FOLFOX regimen at the conventional biweekly schedule. Prevention or cure is one option for which carbamazepine, gabapentin, calcium, and magnesium have already been investigated (7). However, neuromodulatory agents have shown rather disappointing activity in the prevention of oxaliplatin-induced neurotoxicity (8,9).

We developed a new treatment with the mFOLFOX6 regimen administered biweekly for 10-13 consecutive cycles followed by a 3-week rest period, after which treatment was resumed with cycles of tri-weekly mFOLFOX6 administration at standard doses. The present study showed that the standard mFOLFOX6 treatment, followed by tri-weekly mFOLFOX6 maintenance therapy, was associated with a very low incidence of hematological and non-hematological toxicities, including severe neurotoxicity (3.1%), in 32 evaluable metastatic CRC patients. Furthermore, the median RD (9.2 months) and median PFS (8.6 months) were comparable with those usually observed with the mFOLFOX6 regimen (7). The low incidence of severe neurotoxicity observed in our study was clearly related to the planned tri-weekly treatment schedule. The tri-weekly mFOLFOX regimen is not only a way to decrease neurotoxicity but also a new strategy to administer chemotherapy with advantages in costs and quality of life without a deterioration of survival rates.

CONCLUSIONS

The tri-weekly mFOLFOX6 maintenance chemotherapy was associated with a very low incidence of grade 3 neurotoxicity. RD and PFS were comparable to those usually reported in the treatment of metastatic CRC patients. The tri-weekly maintenance strategy may reduce toxicity and maintain efficacy.

TABLE 1. Patient Characteristics.

Characteristics	No. of Patients (%)
Age Median, years	62
Range, years	39-77
Gender	
Male	14 (48.3)
Female	15 (51.7)
Primary tumor Colon	14 (48.3)
Site Rectum	15 (51.7)

TABLE 2. Response to treatment (n=29).

Complete Response	0 (0%)
Partial Response	17 (58.6%)
Stable Disease	10 (34.5%)
Progressive Disease	2 (6.9%)
Response Rate	58.6%
Time to progression	9.4 months (95% CI 8.2-10.5)
Median survival time	23 months (95% CI 19.6-27.8)

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Tumor Response and Negative Distal Resection Margins of Rectal Cancer after Hyperthermochemoradiation Therapy

SOICHI TSUTSUMI[1], YUICHI TABE[1], TAKAAKI FUJII[1], SATORU YAMAGUCHI[1], TOSHINAGA SUTO[1], REINA YAJIMA[1], HIROKI MORITA[1], TOSHIHIDE KATO[1], MARIKO SHIOYA[2], JUN-ICHI SAITO[2], TAKAYUKI ASAO[1], TAKASHI NAKANO[2] and HIROYUKI KUWANO[1]

Departments of [1]General Surgical Science and [2]Radiation Oncology, Gunma University, Graduate School of Medicine, Maebashi, Gunma, Japan

Abstract. *Background: The safety of regional hyperthermia has been tested in locally advanced rectal cancer. The aim of this study was to assess the effects of shorter distal margins on local control and survival in rectal cancer patients who were treated with preoperative hyperthermochemoradiation therapy (HCRT) and underwent rectal resection by using the total mesorectal excision (TME) method. Patients and Methods: Ninety-three patients with rectal adenocarcinoma who received neoadjuvant HCRT (total radiation: 50 Gy) were included in this study. Surgery was performed 8 weeks after HCRT, and each resected specimen was evaluated histologically. Length of distal surgical margins, status of circumferential margins, pathological response, and tumor node metastasis stage were examined for their effects on recurrence and survival. Results: Fifty-eight (62.4%) patients had tumor regression, and 20 (21.5%) had a pathological complete response. Distal margin length ranged from 1 to 55 mm (median, 21 mm) and did not correlate with local recurrence (p=0.57) or survival (p=0.75) by univariate analysis. Kaplan-Meier estimates of recurrence-free survival and local recurrence for the <10 mm versus ≥10 mm groups were not significantly different. Positive circumferential margins and failure of tumors to respond were unfavorable factors in survival. Conclusion: Distal resection margins that are shorter than 10 mm but are not positive appear to be equivalent to longer margins in patients who undergo HCRT followed by rectal resection with TME. To improve the down-staging rate, additional studies are needed.*

Cancer of the lung and bronchus, prostate, and colorectum in men and of the lung and bronchus, breast, and colorectum in women continue to be the most common fatal type of cancer (1). Colorectal cancer alone is expected to account for 9% (26,580) of all male and 9% (24,790) of all female cancer deaths in 2010. More than one-third of colorectal carcinomas occur in the rectum. An important concern in rectal cancer is a high local recurrence rate, as opposed to that in colon cancer. Current guidelines from the National Comprehensive Cancer Network (2) recommend that all patients with clinical stage II/III rectal cancer should be treated with preoperative chemoradiation followed by total mesorectal excision (TME). In locally advanced rectal cancer, the addition of 5-fluorouracil (5-FU) to preoperative radiotherapy has been shown to improve the pathological complete response rate, tumor down-staging, and locoregional control compared with radiotherapy alone (3). Hyperthermia is a procedure that involves heating tissues to a high temperature ranging from 41 to 43°C. This therapy has been combined with radiotherapy and/or chemotherapy for many years, with remarkable success in treating advanced and recurrent cancer. Hyperthermia affects cells in the S phase, inhibits sub-lethal damage repair, and improves oxygenation, making it an attractive therapy to combine with radiation and/or chemotherapy in the hopes of synergy (4). A previous study reported the additional effect of hyperthermia over preoperative radiation alone without any increase in adverse effects (5). Local hyperthermic therapy in combination with radiation has been shown to be less invasive; therefore, the use of local hyperthermia with radiation for local advanced rectal cancer has been recommended as a preoperative therapy.

Sphincter-preserving ultra-low anterior resection (LAR) is preferred to abdominoperitoneal resection (APR) with permanent colostomy for tumors located at least 2 cm proximal of the anal sphincter complex. Previous studies have shown that close distal margins are associated with an increased risk of mucosal recurrence and overall cancer recurrence (6). We have been conducting a clinical trial of regional hyperthermia in

Correspondence to: Soichi Tsutsumi, MD, FACS, Department of General Surgical Science, Gunma University, Graduate School of medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan. Tel: +81 272208224, Fax: +81 272208230, e-mail: chuchumi@showa.gunma-u.ac.jp

Key Words: Rectal cancer, hyperthermo-chemo-radiation therapy, HCRT, distal margins, tumor response.

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combination with chemoradiotherapy (hyperthermochemo-radiation therapy; HCRT) by using chronochemotherapy (7) for locally advanced rectal cancer (8). The advantages of preoperative HCRT include tumor down-staging, improved resectability, overall survival, and increased anal sphincter preservation (9). The aim of this study was to assess the effects of shorter distal margins on local control and survival in rectal cancer patients who were treated with preoperative HCRT and underwent rectal resection by using the TME method.

Patients and Methods

Patients and therapeutic strategy. Between January 2004 and March 2011, 93 patients with proven rectal adenocarcinoma who underwent HCRT followed by surgery were included in this study. During the diagnostic work-up, all patients underwent staging for distant metastases with computed tomography of the abdomen and thorax. T Stage was determined by magnetic resonance imaging, especially T2-weighted imaging before and after HCRT. The extent and location of the tumor were classified according to the TNM (10).

Preoperative HCRT. All patients in this study underwent preoperative HCRT at the Department of Radiology and Radiation Oncology, Gunma University Hospital. The radiation treatment was delivered by 10-MV x-rays through a three-field box technique. The clinical target volume encompassed the primary tumor and the entire mesorectal tissue. The total radiation dose was 50 Gy, with daily fractions of 2.0 Gy on 5 consecutive days per week. Chemotherapy consisted of 5-FU (250 mg/m^2 per day) and levofolinate calcium (25 mg/m^2 per day) administered by continuous infusion at night for 5 days a week in the 1st, 3rd, and 5th weeks of radiation. Two to five hyperthermia sessions were performed once a week with 8 MHz radiofrequency capacitive heating equipment (Thermotron-RF 8, Yamamoto Vinita Co., Ltd., Japan).

Surgery and postoperative therapy. Rectal resection was performed using the principles of TME 8 weeks after the completion of HCRT. A complete 6-month course of adjuvant 5-FU-based chemotherapy was typically recommended for all medically fit patients completing HCRT and curative surgery. The majority of the patients received oral 5-FU/leucovorin.

Pathology. Each resected specimen was examined for histological changes after HCRT according to the histological criteria of the Japanese Classification of Colorectal Carcinoma (11). Grades were assigned according to the amount of necrosis, degeneration, and lytic change of the tumor in the estimated total amount of the lesion (12). Grading of the histopathological response was performed by pathologists. The distal margin was defined as the gross distance between the distal edge of the tumor or post-treatment fibrosis, if present, and the distal mucosal resection margin.

Results

Patients' characteristics. Ninety-three patients with lower third rectal cancer were included in this study; the median age was 64 years. Sixty-eight patients were males, and 25 patients were females, with a male-to-female ratio of 2.7: 1. Patient characteristics are shown in Table I. All patients tolerated this regimen without hematological toxicity. The non-hematological toxicities observed were diarrhea in one patient and anorexia in one patient, both with grade 3 cancer. When the clinical pretreatment stage was compared with the pathologic results, down-staging of the T and N stages was possible in 51 (45.2%) and 54 patients (58.1%), respectively. The overall down-staging rate, including both the T and N stages, was 63.4% (59/93). Significant down-staging estimated in the primary lesion, in which tumors were undetectable by MRI and colonoscopy and negative results were obtained from biopsies, occurred in 47 patients (50.5%). Anterior resection was performed with colorectal anastomosis using a double-stapler device in 55 (59.1%) patients and with coloanal anastomosis using the hand-sewn technique in 21 (22.6%) patients. Abdominoperineal resection was performed in 17 (18.3%) patients. The overall sphincter preservation rate in the present study was 81.7% (76 out of 93 patients).

Table I. *Patients' characteristics.*

Characteristic	No. of cases (%)
Age, years	
Median (range)	64 (43-85)
Gender	
Male	68 (73.1)
Female	25 (26.9)
Stage	
II	39 (41.9)
III	50 (53.8)
IV	4 (4.3)
Surgical technique	
APR	17 (18.3)
LAR	76 (81.7)
Sphincter-preserving rate	81.7%

APR, Abdominoperitoneal resection; LAR, low anterior resection.

The pathological diagnoses obtained from surgical specimens are shown in Table II. Pathological complete response (pCR) of the primary tumor (Figure 1) and lymph nodes on the pathological specimen was observed in 20 patients (21.5%), and one patient showed pCR of the primary tumor but had residual tumor cells in the regional node. All patients had pathologically negative distal resection margins. Patients with pCR after HCRT had no better outcome than did those without pCR (Table II). pCR after HCRT might not be indicative of a prognostically favorable biological tumor profile. For the LAR specimen, the distal resection margins ranged from 1 to 55 mm (median 21 mm), not including the anastomotic staple rings. Fourteen (15.1%) patients who underwent LAR had distal resection margins of <10 mm. Seven (7.5%) patients were found to have positive circumferential margins with the pelvic side wall.

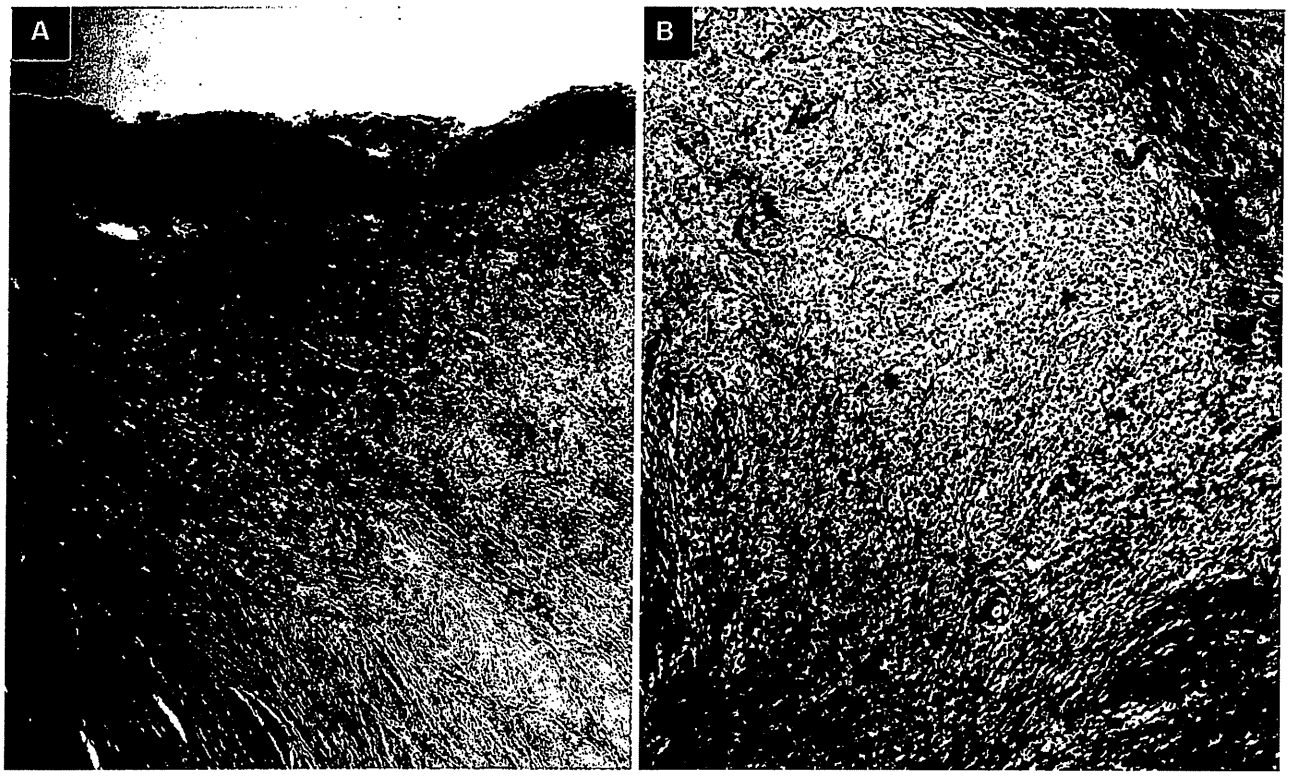

Figure 1. *Microscopic evaluation of tumor response. A: Low magnification (×25) image showing the ulcer at the tumor bed site. B: Higher magnification (×100) image deep in the wall of the fibrosis shows a high number of lymphocytes and fibroblasts scattered throughout the scar tissue.*

The median follow-up was 37 months (range 4-81 months) for all patients, and 84 patients were still alive at the time of writing. A distal margin length shorter than 10 mm did not correlate with local recurrence (p=0.57) or survival (p=0.73) by univariate analysis. Kaplan-Meier estimates of recurrence-free survival and local recurrence for the <10 mm *versus* >10 mm groups were not significantly different (Figure 2). However, positive circumferential margin and down-staging were related to overall survival (Figures 3 and 4).

Discussion

Whether LAR or APR is performed, ensuring that the intact rectum and mesorectum are removed with clear surgical margins is immensely important to prevent the local recurrence of rectal cancer. Preoperative chemoradiation can potentially increase the feasibility of sphincter-preserving resections by reducing the tumor volume and by defending against local tumor extensions (13). Given the additional effects of hyperthermia on chemoradiation therapy, HCRT may compensate for the narrow circumference and distal resection margins. The rate of

Table II. *Comparison of clinicopathologic variables.*

Distal resection margin	<10 mm n=14	≥10 mm n=79	*p*-value
Local recurrence	1 (7.1%)	3 (3.8%)	0.5745
Survival	13 (92.9%)	71 (89.9%)	0.7313
Pathologic circumferential resection margin	– n=86	+ n=7	*p*-value
Local recurrence	3 (3.5%)	1 (14.3%)	0.1795
Survival	79 (91.9%)	5 (71.4%)	0.029
Pathologic complete response	pCR (–) n=73	pCR (+) n=20	*p*-value
Local recurrence	4 (2.3%)	0 (0%)	0.2896
Survival	70 (91.9%)	20 (71.4%)	0.1006
Down-staging	– n=34	+ n=59	*p*-value
Local recurrence	3 (8.8%)	1 (1.7%)	0.1049
Survival	27 (79.4%)	57 (96.6%)	0.0187

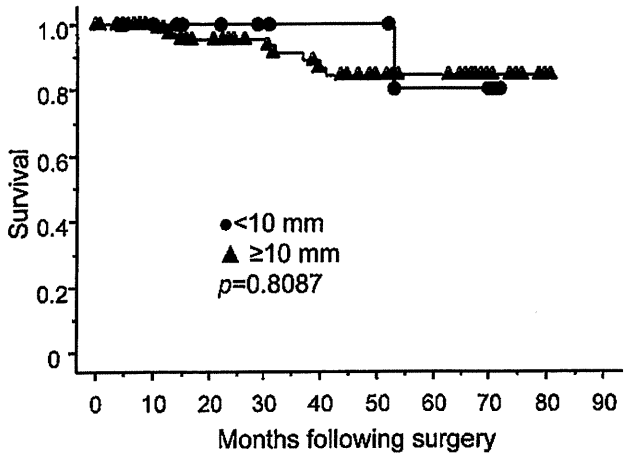

Figure 2. *Kaplan-Meier distribution of overall survival for patients with <10 mm distal margins versus those with ≥10 mm distal margins.*

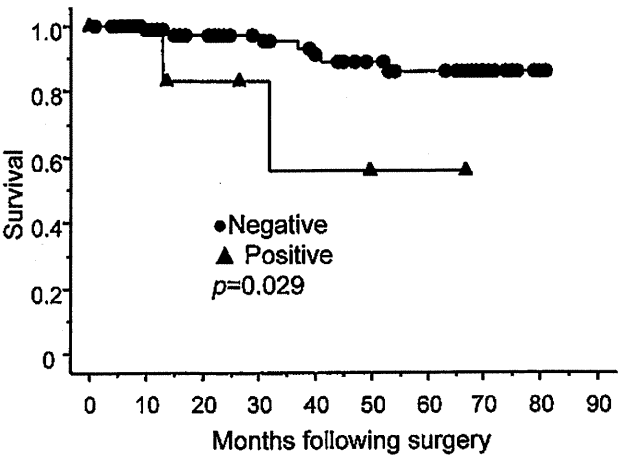

Figure 3. *Kaplan-Meier distribution of overall survival for patients with negative circumferential margins versus those with positive circumferential margin.*

pCR after neoadjuvant chemoradiation therapy ranged from 10 to 16% in various series examined in a review (14). Recent efforts incorporating newer cytotoxic and molecularly targeted agents into chemoradiation therapy regimens have been reported. Large randomized trials showed that the addition of weekly oxaliplatin to fluoropyrimidine-based chemoradiation led to an increase in grade 3/4 toxicity but no difference in pCR rates. Early phase trials evaluating the anti-epidermal growth factor receptor antibody cetuximab in combination with chemoradiation reported modest pCR rates of 5 to 12%. In this study, the pCR rate was 21.5%, which compares favorably with that observed in the other reports. Moreover, our study showed a greater reduction of adverse effects through the use of chronochemotherapy (8).

Tumor distance from the anal verge was significantly greater after HCRT, thus it was possible to carry out sphincter-preserving surgery in a larger proportion of the patients. The close distal resection margin, even if it was shorter than 10 mm, was not related to local recurrence and survival in this study. A positive circumferential margin was also not related to local failure (Table II). Obtaining negative distal and circumferential margins remains a goal of rectal cancer surgery after HCRT. The malignant potential and behavior of tumor after HCRT might be different from the pretreatment status. Further investigations are needed in order to archive better individual oncologic results.

Down-staging in this study was a good predictor of outcome. Down-staging oncological treatment has not been viewed as an additional therapy, and there is no evidence-based protocol to follow if the tumor fails to regress or increases in size after HCRT. Trials of chemotherapy with

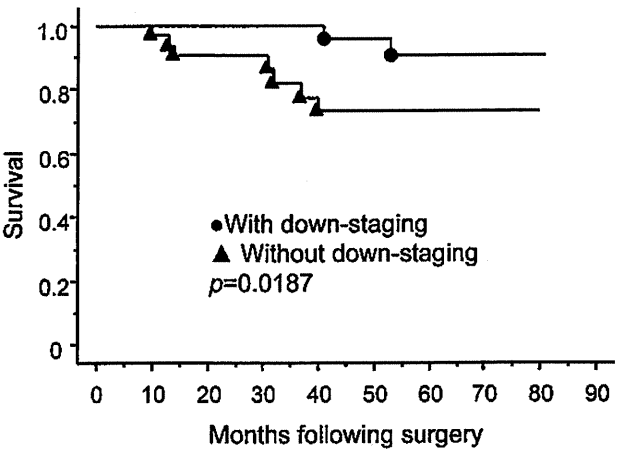

Figure 4. *Kaplan-Meier distribution of overall survival for patients with down-staging versus those without down-staging.*

new biologic agents in a preoperative setting for patients whose disease fails to down-stage after HCRT are needed. New techniques for rectal cancer surgery also need to be investigated. However, a prospective randomized study that inspects the adequacy and safety of the distal and circumferential resection margins would be difficult to set up. The limitations of our study include the small number of patients and the short follow-up period. The results of our study are encouraging in terms of the rate of down-staging, pCR, and sphincter-preserving surgery. The favorable results of our study might be due to additional hyperthermia with chemoradiation therapy. Further investigations to improve the down-staging rate of HCRT for rectal cancer are required.

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症例報告

メシル酸イマチニブによる術前化学療法を施行しpCRが得られた胃原発GIST局所再発の1切除例

桐生厚生総合病院外科[1)]
同　病理部[2)]
日野市立病院外科[3)]
群馬大学大学院病態総合外科学[4)]

木村　明春[1)]　平松　聖史[1)]　櫻川　忠之[3)]　土屋　智敬[1)]
尾辻　英彦[1)]　前田　隆雄[1)]　田中　　寛[1)]　吉田カツ江[2)]
待木　雄一[1)]　桑野　博行[4)]

症例は78歳の男性で，2004年11月，胃gastrointestinal stromal tumor（以下，GISTと略記）に対して噴門側胃切除術を施行した．免疫組織学的検査でKITおよびCD34が陽性，核分裂像が高度であり高リスクのGISTと診断されたため，術後より1年9か月の間，イマチニブ(400mg/日)の投与を行った．2008年11月の腹部造影CTで残胃に局所再発を認めたため，イマチニブの投与を再開した．投与3か月後の腹部造影CTでは腫瘍の縮小を認め，再手術を施行した．切除標本の病理組織学的検査では，腫瘍細胞は認めず，血管腫様の組織を認めるのみでpathological complete response（以下，pCRと略記）と判断した．横隔膜と肝外側区への浸潤を伴う再発胃GISTに対して，イマチニブによる術前化学療法を施行後に切除し，pCRが得られた希少な症例を経験したため報告する．

はじめに

GISTは消化管に発生する間葉系腫瘍のうち，平滑筋細胞，神経細胞への分化を示さず，免疫染色検査でKIT陽性を示すものとされる[1)]．

これまで治療抵抗性であったGISTの再発例，転移例に対して，現在はメシル酸イマチニブ(以下，イマチニブと略記)の投与が第一選択となっている[2)]．イマチニブは高い奏効率が報告されているが，単独での治癒は困難であり，長期投与による耐性や休薬後の腫瘍増大などの問題も指摘されている．そのため，切除可能な場合には外科的切除が必要と考えられ，外科的治療の介入によって，治療効果の向上が期待される．今回，我々は局所再発を来たした胃GISTに対してイマチニブを投与後に切除し，pCRが得られた症例を経験したので報告する．

症　例

患者：78歳，男性

主訴：特になし

既往歴：大腸癌．

現病歴：2004年11月，穹隆部の胃GISTに対して噴門側胃切除術（食道残胃吻合）を施行した．腫瘍径は13cmで，病理組織学的検査では紡錘形細胞の増殖を認め，免疫組織化学的検査ではKITおよび

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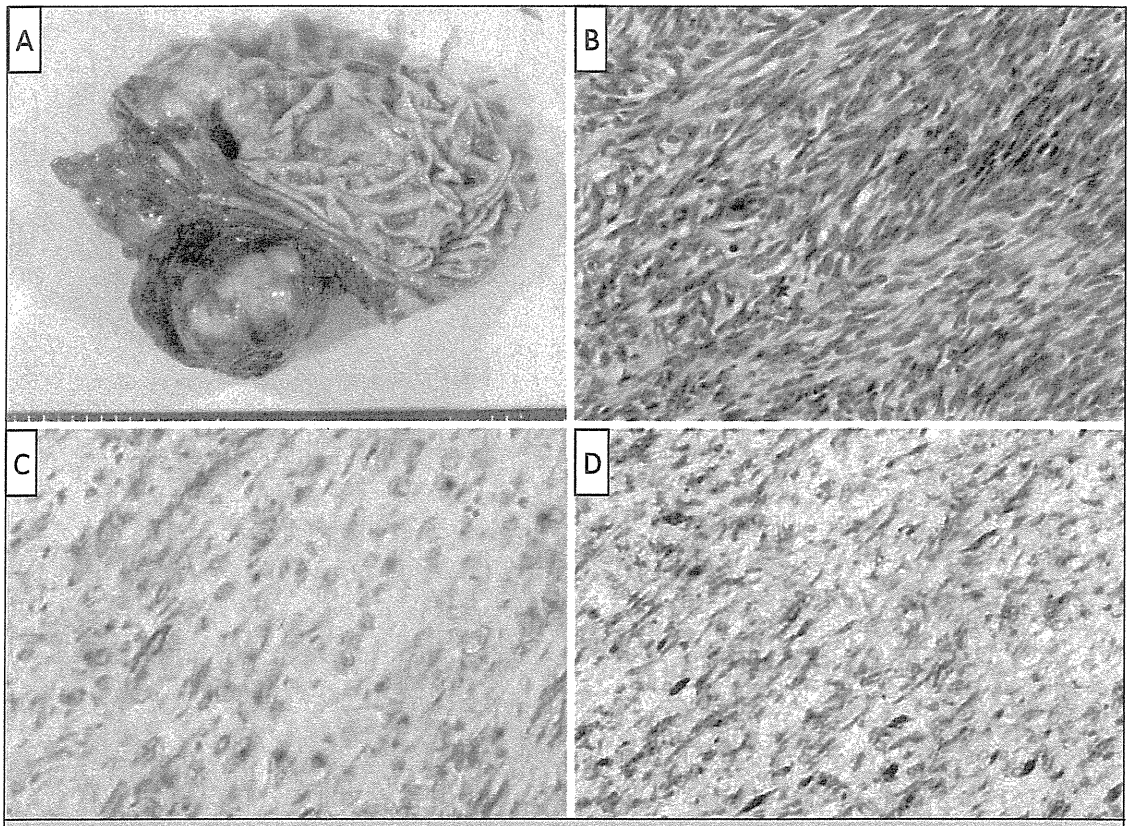

Fig. 1 Macroscopic and histological findings of the initially resected tumor of the stomach. A: The tumor measured 13 cm in diameter. B: Proliferation of spindle cell. C and D: The tumor cells were positive for c-kit (C) and CD34 (D).

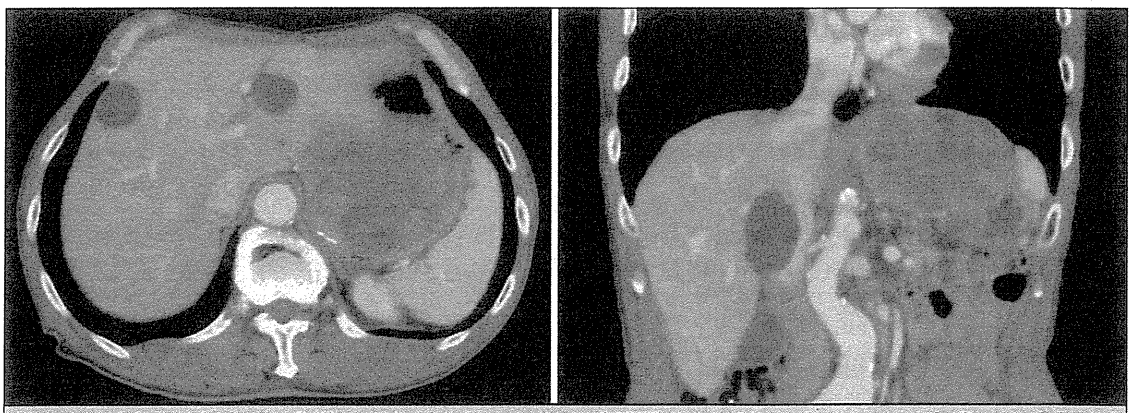

Fig. 2 Abdominal enhanced CT revealed local recurrence of GIST, invaded lateral segment of the liver and the diaphragm.

CD34 が陽性であった(**Fig. 1**). また核分裂像も高度であり，高リスクの GIST であったため，術後から 1 年 9 か月の間，イマチニブ（400mg/日）の投与を行った.

以後，定期的に外来で経過観察を行い，2008 年 3 月に撮影した CT では明らかな再発所見を認めなかった. 術後より約 4 年経過した 2008 年 11 月の腹部造影 CT で残胃に腫瘍性病変を認めた. 腫瘍は径 8.5×7.2cm で前回手術時の staple line を含め残胃を中心に存在しており，局所再発と診断した. 腫瘍は肝外側区域および食道裂孔を含め左横隔膜へ広範に浸潤陽性と診断された（**Fig. 2**）. 上部消化管内視鏡検査では胃体部を中心に粘膜下腫瘍様の隆起を認め，胃内腔を圧排していた. 根治切除を行うには広範囲