Table 2. Postoperative complications and mortality in perforated and uncomplicated cancers

	No. (%)		
	Perforating cancer (n = 22)	Uncomplicated cancer (n = 826)	P value
Mortality	2 (9)	8 (1)	0.0255
Complications			
Total	11 (50)	193 (23.4)	0.0076
Anastomotic leak	2 (9.1)	22 (2.7)	0.1257
Abdominal abscess	3 (13.6)	3 (0.4)	0.0003
Wound infection	6 (27.3)	50 (6)	0.0021
Sepsis	2 (9.1)	3 (0.4)	0.0061
Respiratory disturbance	3 (13.6)	19 (2.3)	0.0170
Cardiovascular disturbance	0 (0)	13 (1.6)	>0.05
Others	0 (0)	83 (10)	>0.05

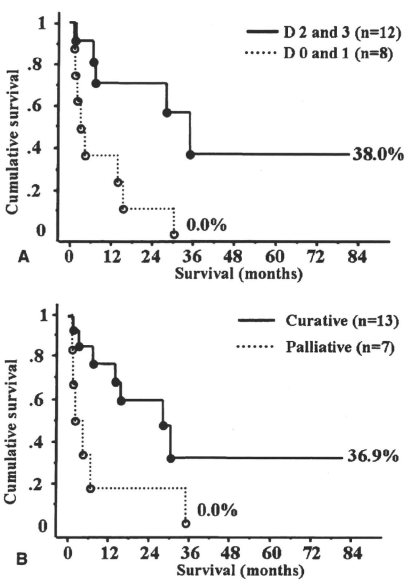

Fig. 1. **A** Survival curves for patients who underwent potentially radical D2 or D3 lymph node dissection (n = 12), or nonradical D0 or D1 lymph node dissection (n = 8) (P = 0.0045, log-rank test). **B** Survival curves for patients who underwent either a potentially curative resection (n = 13) or a palliative resection (n = 7) (P = 0.0108, log-rank test)

operation, resulted in 15 patients who had a mean survival of 28 months and a 5-year survival rate of 32%. Excluding postoperative mortality, the overall survival was 38% following potentially radical lymph node dissection (so-called D2 or D3), as compared with 0% in patients who underwent nonradical lymph node dissection (so-called D0 or D1), respectively (P = 0.0045). The patients who underwent a potentially curative resection had a significantly better crude 5-year survival rate than those who underwent palliative resection (36.9% vs 0%; P = 0.0108).

Discussion

Perforation has been reported to occur in 3%–9% of colorectal cancers,[2,3,9,10] although the incidence in our study was slightly lower, at 2.6%. Perforated colorectal cancer is generally associated with a low survival rate and high postoperative mortality.[2-4,6] Its outcome is worse than that of other complicated presentations including obstruction and bleeding,[6] the reasons for which are multifactorial. The survival and outcome of these patients depends on their general condition, the severity of sepsis, if there is locally advanced malignancy, and the presence of metastatic disease at the time of perforation.[4,9] The operative mortality was 9% in our series, which compares favorably to previously reported mortality rates of 30%–43%.[2,4,5,9] It is not surprising that the postoperative morbidity and mortality rates are higher in these patients than in those who undergo elective surgery for colorectal cancer. Many of these patients are elderly, with a mean age of 71.8 years in our study, and therefore likely to suffer from cardiovascular or respiratory dysfunction before their acute admission.[11] Moreover, advanced age tends to delay diagnosis, and peritonitis may develop rapidly in this population. Under these circumstances, the timing of the diagnosis may determine survival.

Traditionally, Hartmann's procedure, or resection and double-barrel colostomy, was the surgical procedure of choice, but now one-staged procedures are often performed with a significant survival advantage.[3,5,10] Most (96%) of the patients in this series had

left colon lesions and 15 (68%) underwent Hartmann's procedure, or resection and colostomy, while only four underwent resection and primary anastomosis. A curative resection was possible in 69% of the patients, which compares favorably with historical rates of resection (60%–92%).[12,13] Our rationale for attempting potentially curative resection was supported by results reported by others.[10,14] These researchers found that the type of surgical treatment played an important role in prognosis. Their retrospective review of patients presenting with obstructing or perforated cancers revealed a significant decrease in hospital mortality and improvement in both 3-year and 5-year survival for those patients who underwent a potentially curative surgery versus a staged drainage procedure.

The crude 5-year survival for patients with perforation of colorectal cancer has been reported to range from 7% to 44%.[3,9] Although the overall 5-year survival rate in this study population was 17.4%, when adjusted to exclude operative mortality and patients with metastatic disease at the onset, it increased to 32%.

The en bloc curative operation, potentially including radical lymph node dissection, or so-called D2 or D3, may be warranted, in accordance with other reports.[10,14,15] In the present series, patients with stage IV disease at diagnosis and those who died within the 30-day postoperative period were excluded from the total 15 patients who underwent potentially curative resection, yielding 13 patients. This group of 13 patients was found to have a mean survival of 30 months and a 5-year survival rate of 36.9%; significantly better than that of those who underwent a noncurative resection (0%, $P = 0.0108$). This 5-year survival rate of 36.9% compared favorably with data on patients with colorectal cancer reported from Japan and with the reported 13.2% 5-year survival rate of patients with stage IV disease.[8]

The results of the present study show that patients presenting with perforated cancers tend to have advanced disease, a low curative resection rate, a high postoperative mortality rate, and poor overall survival. Hence, intensive treatment, including sepsis management and surgery, is usually warranted. Adjuvant chemotherapy is effective for advanced colorectal cancer[5] and may therefore be routinely recommended for patients with perforated colorectal cancer.

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Short Communication

Mexiletine Reverses Oxaliplatin-Induced Neuropathic Pain in Rats

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Abstract. Oxaliplatin is a platinum-based chemotherapy drug characterized by the development of acute and chronic peripheral neuropathies. Mexiletine, an orally available Na^+-channel blocker, has widely been used in patients with chronic painful diabetic neuropathy. In the present study, we examined the effect of mexiletine on oxaliplatin-induced neuropathic pain in rats. Mexiletine (100, but not 10 and 30, mg/kg, p.o.) completely reversed both mechanical allodynia and cold hyperalgesia induced by oxaliplatin (4 mg/kg, i.p., twice a week). Lidocaine (30, but not 3 and 10, mg/kg, i.p.) also significantly relieved both pain behaviors. These results suggest that mexiletine may be effective in relieving the oxaliplatin-induced neuropathic pain clinically.

Keywords: mexiletine, oxaliplatin, neuropathic pain

Oxaliplatin, a third-generation platinum-based chemotherapy drug, is a key drug in the treatment of colorectal cancer. Unlike other platinum compounds, oxaliplatin induces an acute painful neuropathy, which appears soon after administration (1). The patients suffer from extremity and perioral paresthesias and in particular from severe cold hypersensitivity. After multiple cycles the patients develop a clinically different peripheral neuropathy that is characterized by a sensory axonal nerve damage closely resembling that induced by cisplatin. This chronic neuropathy can become very disabling and is, in fact, often a dose-limiting toxicity. For this reason, peripheral neuropathy associated with the administration of oxaliplatin is a major clinical problem in chemotherapy.

Mexiletine, an orally available Na^+-channel blocker, has been reported to be effective on chronic painful diabetic neuropathy in clinical trial (2), and it is prescribed for treating patients with these symptoms. In animal models, acute administration of mexiletine has been reported to relieve the mechanical allodynia in rats treated with vincristine, a chemotherapeutic agent, and streptozotocin-induced diabetic rats (3, 4). No experimental study, however, has been conducted to date to determine the effect of mexiletine on pain behavior in a rat model of oxaliplatin-induced neuropathy. In the present study, we examined the effect of mexiletine on the oxaliplatin-induced mechanical allodynia and cold hyperalgesia after the development of neuropathy in rats.

Male Sprague-Dawley rats weighing 200 – 250 g (Kyudo Co., Saga) were used in the present study. Rats were housed in groups of four to five per cage, with lights on from 08:00 to 20:00 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 IASP Committee for Research and Ethical Issues guidelines for animal research (5).

Oxaliplatin (Elplat®) was obtained from Yakult Co., Ltd. (Tokyo). Mexiletine hydrochloride was purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Lidocaine (Xylocaine® 2% for intravenous injection) was obtained from Astra Zeneca K.K. (Osaka). Oxaliplatin was dissolved in 5% glucose solution. The vehicle-treated rats were injected with 5% glucose solution. Oxaliplatin (4 mg/kg) or vehicle was injected intraperitoneally (i.p.) twice a week for 4 weeks (Days 1, 2, 8, 9, 15, 16, 22, and 23). Mexiletine was dissolved in sterile water and administered orally. Lidocaine was dissolved in saline and administered i.p. The doses of these drugs were chosen based on previous reports (3, 4, 6). Behavioral tests were performed blindly with respect to drug administration.

The mechanical allodynia was assessed by the von Frey test. Rats were placed in a clear plastic box

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(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, UK) of 1 – 15 g bending force were applied to the midplantar skin of each hind paw with each application held for 6 s. Fifty percent paw withdrawal thresholds were determined by up-down methods (7).

The cold hyperalgesia was assessed by the acetone test described by Flatters and Bennett (8). 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. A 50-μL aliquot of acetone (Wako Pure Chemical, Ltd., Osaka) was sprayed onto the plantar skin of each hind paw three times with a Micro Sprayer® (Penn Century Inc., Philadelphia, PA, USA), and the number of withdrawal responses was counted for 40 s from the start of the acetone spray.

We confirmed the incidence of mechanical allodynia and cold hyperalgesia on Days 24 and 3, respectively. We carried out the drug evaluation on the next day. In the case of mexiletine, the von Frey and acetone tests were performed immediately before (0 min) and at 60, 120, and 180 min after administration. In the case of lidocaine, the von Frey and acetone tests were performed immediately before (0 min) and at 30, 60, and 120 min after administration.

Values were expressed as the mean ± S.E.M. The values were analyzed by Student's *t*-test or one-way analysis of variance (ANOVA) followed by the Tukey-Kramer's post-hoc test (StatView; Abacus Concepts, Berkeley, CA, USA) to determine differences among the groups. A probability level of $P < 0.05$ was accepted as statistically significant.

Oxaliplatin (4 mg/kg, i.p.) significantly reduced the 50% paw withdrawal threshold compared with the vehicle in the von Frey test on Day 24 ($P < 0.01$, Figs. 1A and 2A). Oxaliplatin at the same dose significantly increased the number of withdrawal responses compared with vehicle in the acetone test on Day 3 ($P < 0.01$, Figs. 1B and 2B). The incidence of mechanical allodynia and cold hyperalgesia was 92% and 81%, respectively. Acute administration of mexiletine (100 mg/kg, p.o.) completely reversed the reduction of 50% paw withdrawal threshold by oxaliplatin at 60 min after administration in the von Frey test ($P < 0.01$, Fig. 1A). Moreover, mexiletine (100 mg/kg, p.o.) completely reversed the increase of number of withdrawal responses by oxaliplatin at 60 and 120 min after administration in the acetone test ($P < 0.05$, Fig. 1B). These effects of mexiletine disappeared by 180 min after administration. Similarly, acute administration of lidocaine (30 mg/kg, i.p.) significantly inhibited the reduction of 50% paw withdrawal threshold by oxaliplatin at 30 min after administration in the von Frey test ($P < 0.05$, Fig. 2A). Moreover, lidocaine (3, 10, and 30 mg/kg, i.p.) significantly inhibited the increase of number of withdrawal responses by oxaliplatin at 30 min after administration in the acetone test ($P < 0.01$, Fig. 2B). These effects of lidocaine had disappeared by 120 min after administration. In addition, mexiletine (100 mg/kg, p.o.) and lidocaine (30 mg/kg, i.p.) had no effect on the 50% paw withdrawal threshold in the von Frey test and the number of withdrawal responses in the acetone test in intact rats (data not shown).

Our data in this study revealed that acute administration of mexiletine completely reversed both mechanical

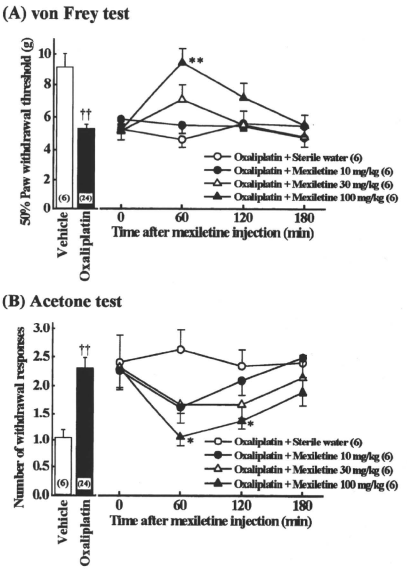

Fig. 1. Effect of mexiletine on mechanical allodynia in the von Frey test (A) and cold hyperalgesia in the acetone test (B) in oxaliplatin-treated rats. Oxaliplatin (4 mg/kg) was administered i.p. twice a week for 4 weeks (Days 1, 2, 8, 9, 15, 16, 22, and 23). We confirmed the incidence of mechanical allodynia and cold hyperalgesia on Days 24 and 3, respectively. We carried out the drug evaluation on the next day. Mexiletine was administered orally. The von Frey and acetone tests were performed immediately before (0 min) and at 60, 120, and 180 min after administration. Number of animals is shown in parenthesis. Values are expressed as the mean ± S.E.M. $^{\dagger\dagger}P < 0.01$, compared with vehicle; $^{*}P < 0.05$, $^{**}P < 0.01$, compared with oxaliplatin alone.

allodynia and cold hyperalgesia induced by oxaliplatin. Mexiletine has widely been used in the treatment of chronic painful diabetic neuropathy. It has also been reported that mexiletine produced no major adverse events and was superior to placebo to relieve neuropathic pain in controlled clinical trials (9). Taken together, the present results suggest that mexiletine is useful as a therapeutic drug for oxaliplatin-induced neuropathic pain if it is used with caution as needs arise.

Similarly, lidocaine, another Na^+-channel blocker, significantly relieved both pain behaviors. Ling and colleagues (10) have reported that single intravenous administration of lidocaine relieved the oxaliplatin-induced cold allodynia in rats. Our finding is essentially consistent with the previous finding. Moreover, we found that mexiletine and lidocaine at the effective dose had no effect on pain behavior in intact rats. Therefore, the ameliorative effects of mexiletine and lidocaine were not attributable to non-specific sedative effects or a deficit of motor function. These findings suggest that the reduced pain behavior by Na^+-channel blockers reflects a therapeutic effect on oxaliplatin-induced neuropathic pain. Asano et al. (11) reported that mexiletine at the dose of 20 mg/kg did not affect pain-related responses in normal mice. They also indicated that activation of the descending β-endorphinergic system is involved in the antinociceptive effect of mexiletine. The β-endorphinergic system is generally accepted as an antinociceptive system, which selectively has antinociceptive effect on painful conditions. In the in vitro studies, application of oxaliplatin to dorsal root ganglion (DRG) neurons resulted in an increase of the Na^+ current (12). Interestingly, the effect of oxaliplatin is antagonized by the Na^+-channel blocker carbamazepine (12). Therefore, mexiletine and lidocaine exhibit effective relief on the oxaliplatin-induced neuropathic pain, but may be ineffective in reducing pain-related behaviors in intact rats.

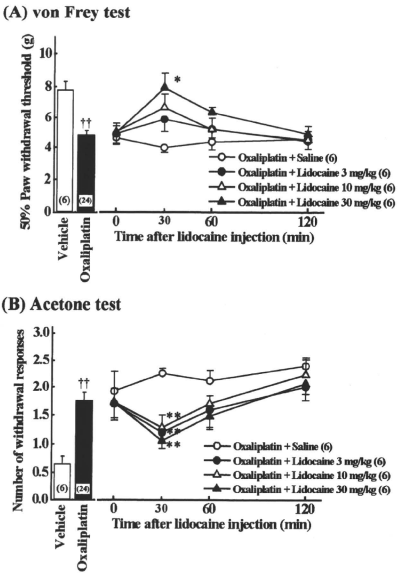

Fig. 2. Effect of lidocaine on mechanical allodynia in the von Frey test (A) and cold hyperalgesia in the acetone test (B) in oxaliplatin-treated rats. Oxaliplatin (4 mg/kg) was administered i.p. twice a week for 4 weeks (Days 1, 2, 8, 9, 15, 16, 22, and 23). We confirmed the incidence of mechanical allodynia and cold hyperalgesia on Days 24 and 3, respectively. We carried out the drug evaluation on the next day. Lidocaine was administered i.p. The von Frey and acetone tests were performed immediately before (0 min) and at 30, 60, and 120 min after administration. Number of animals is shown in parenthesis. Values are expressed as the mean ± S.E.M. $^{\dagger\dagger}P < 0.01$, compared with vehicle; $^{*}P < 0.05$, $^{**}P < 0.01$, compared with oxaliplatin alone.

In the present study, mexiletine reversed mechanical allodynia and cold hyperalgesia to the same degree. Lidocaine also relieved both pain-related behaviors. Recently, we demonstrated that oxalate and platinum metabolite are involved in the cold hyperalgesia and mechanical allodynia, respectively (6). Oxalate alters voltage-gated Na^+ channels (13) and its effect may be involved in the cold hyperalgesia. On the other hand, the mechanical allodynia may be due to the peripheral nerve injury by platinum metabolite. The change in the expression of Na^+ channels is observed after peripheral nerve injury of the rat DRG neurons (14). Taken together with these findings, the present results suggest that mexiletine and lidocaine may reverse the mechanical allodynia and cold hyperalgesia by inhibiting the hyperexcitability of Na^+ channels.

In conclusion, the study presented here demonstrates, for the first time, that acute administration of mexiletine reverses both mechanical allodynia and cold hyperalgesia induced by oxaliplatin in rats.

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MOLECULAR PAIN

RESEARCH Open Access

Involvement of spinal NR2B-containing NMDA receptors in oxaliplatin-induced mechanical allodynia in rats

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Abstract

Background: Oxaliplatin is a platinum-based chemotherapy drug characterized by the development of acute and chronic peripheral neuropathies. The chronic neuropathy is a dose-limiting toxicity. We previously reported that repeated administration of oxaliplatin induced cold hyperalgesia in the early phase and mechanical allodynia in the late phase in rats. In the present study, we investigated the involvement of NR2B-containing N-methyl-D-aspartate (NMDA) receptors in oxaliplatin-induced mechanical allodynia in rats.

Results: Repeated administration of oxaliplatin (4 mg/kg, i.p., twice a week) caused mechanical allodynia in the fourth week, which was reversed by intrathecal injection of MK-801 (10 nmol) and memantine (1 μmol), NMDA receptor antagonists. Similarly, selective NR2B antagonists Ro25-6981 (300 nmol, i.t.) and ifenprodil (50 mg/kg, p.o.) significantly attenuated the oxaliplatin-induced pain behavior. In addition, the expression of NR2B protein and mRNA in the rat spinal cord was increased by oxaliplatin on Day 25 (late phase) but not on Day 5 (early phase). Moreover, we examined the involvement of nitric oxide synthase (NOS) as a downstream target of NMDA receptor. L-NAME, a non-selective NOS inhibitor, and 7-nitroindazole, a neuronal NOS (nNOS) inhibitor, significantly suppressed the oxaliplatin-induced pain behavior. The intensity of NADPH diaphorase staining, a histochemical marker for NOS, in the superficial layer of spinal dorsal horn was obviously increased by oxaliplatin, and this increased intensity was reversed by intrathecal injection of Ro25-6981.

Conclusion: These results indicated that spinal NR2B-containing NMDA receptors are involved in the oxaliplatin-induced mechanical allodynia.

Background

Glutamate is a major excitatory transmitter in the spinal cord and N-methyl-D-aspartate (NMDA) receptors are known to be involved in the painful neuropathy [1,2]. The NMDA receptor antagonist inhibits the pain hypersensitivity in chronic constriction injury (CCI) model. Moreover, the expression of spinal NR2B-containing NMDA receptors is increased with the pain hypersensitivity induced by CCI or chronic compression of the dorsal root ganglia (CCD) [3-6]. Selective NR2B antagonists inhibit mechanical allodynia without causing motor dysfunction in CCI, CCD and spinal nerve-ligated (SNL) neuropathic models [5-8]. In addition, the NR2B subunits are localized to the superficial dorsal horn of the spinal cord [7,9], suggesting a possible involvement in pain transmission. Thus, the NR2B-containing NMDA receptors may play an important role in the neuropathic pain.

Nitric oxide synthase (NOS) as a downstream target of NMDA receptor also contributes greatly to the incidence of neuropathic pain. In the rat CCI model of neuropathic pain, intrathecal injection of non-selective NOS inhibitor L-N^G-nitro-arginine methyl ester (L-NAME) reverses the persistent thermal hyperalgesia [10]. Furthermore, a close correlation between neuronal NOS (nNOS) and neuropathic pain has been documented in CCI model [11].

Oxaliplatin, a third-generation platinum-based chemotherapy drug, has widely been used as a key drug in

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the treatment of colorectal cancer. However, oxaliplatin causes severe acute and chronic peripheral neuropathies. The acute neuropathy includes acral paresthesias and dysesthesia triggered or enhanced by exposure to cold, and it appears soon after administration [12]. After multiple cycles the patients develop the chronic neuropathy that is characterized by a sensory and motor dysfunction. This chronic neuropathy is a dose-limiting toxicity and a major clinical problem in oxaliplatin chemotherapy [13].

Recently, we reported that repeated administration of oxaliplatin induced cold hyperalgesia in the early phase and mechanical allodynia in the late phase in rats [14]. Oxaliplatin is metabolized to oxalate and dichloro(1,2-diaminocyclohexane)platinum [Pt(dach)Cl_2] [15]. We demonstrated that oxalate and platinum metabolite are involved in the cold hyperalgesia and mechanical allodynia, respectively [14]. Oxalate alters voltage-gated Na^+ channels [16] and its effect may be involved in the cold hyperalgesia. On the other hand, the mechanical allodynia may be due to the peripheral nerve injury by platinum metabolite. However, whether the NR2B-containing NMDA receptors are involved still remains largely unclear. In the present study, we investigated the involvement of NR2B-containing NMDA receptors in the oxaliplatin-induced mechanical allodynia in rats.

Results

Effects of NMDA receptor antagonists on oxaliplatin-induced mechanical allodynia

Oxaliplatin (4 mg/kg, i.p., twice a week for 4 weeks) significantly reduced the paw withdrawal thresholds compared with the vehicle in the von Frey test on Day 24 ($P < 0.01$, Figure 1). Acute administration of a NMDA receptor antagonist MK-801 (10 nmol, i.t.) completely reversed the reduction of paw withdrawal threshold by oxaliplatin at 30 min after administration ($P < 0.05$, Figure 1A). Similarly, acute administration of another NMDA receptor antagonist memantine (1 μmol, i.t.) completely reversed the reduction of paw withdrawal threshold by oxaliplatin at 30 min after administration ($P < 0.05$, Figure 1B). These effects of MK-801 and memantine disappeared by 120 min after administration. In addition, MK-801 (10 nmol, i.t.) and memantine (1 μmol, i.t.) had no effect on the paw withdrawal thresholds in intact rats (data not shown).

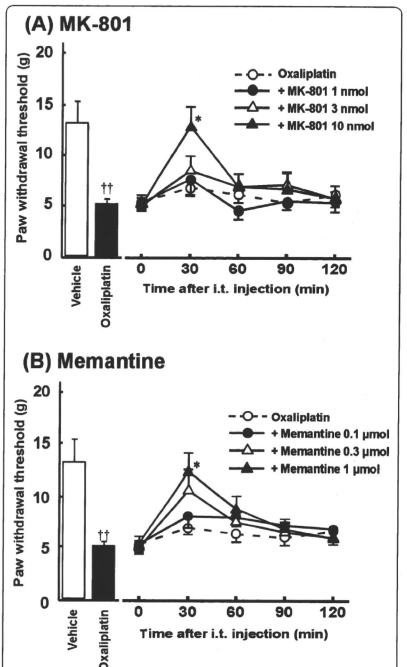

Figure 1 Effects of MK-801 (A) and memantine (B) on oxaliplatin-induced mechanical allodynia in the von Frey test. Oxaliplatin (4 mg/kg) was administered i.p. twice a week for 4 weeks (Days 1, 2, 8, 9, 15, 16, 22 and 23). We confirmed the incidence of mechanical allodynia on Day 24. We carried out the drug evaluation on the next day. MK-801 (1-10 nmol/body) and memantine (0.1-1 μmol/body) were administered intrathecally. The von Frey test was performed immediately before (0 min) and at 30, 60, 90 and 120 min after administration. Values are expressed as mean ± SEM of 6-10 animals. $^{††}P < 0.01$ compared with vehicle, $^{*}P < 0.05$ compared with oxaliplatin alone.

Effects of NR2B antagonists on oxaliplatin-induced mechanical allodynia

Acute administration of a selective NR2B antagonist Ro 25-6981 (300 nmol, i.t.) significantly inhibited the reduction of paw withdrawal threshold by oxaliplatin at 30 and 60 min after administration ($P < 0.01$: 30 min, $P < 0.05$: 60 min, Figure 2A). Similarly, acute administration of another NR2B antagonist ifenprodil (50 mg/kg, p.o.) significantly inhibited the reduction of paw withdrawal threshold by oxaliplatin at 30 and 60 min after administration ($P < 0.01$: 30 min, $P < 0.05$: 60 min, Figure 2B). These effects of Ro 25-6981 and ifenprodil disappeared by 120 min after administration. In addition, Ro 25-6981 (300 nmol, i.t.) and ifenprodil (50 mg/kg, p.o.) had no effect on the paw withdrawal thresholds in intact rats (data not shown).

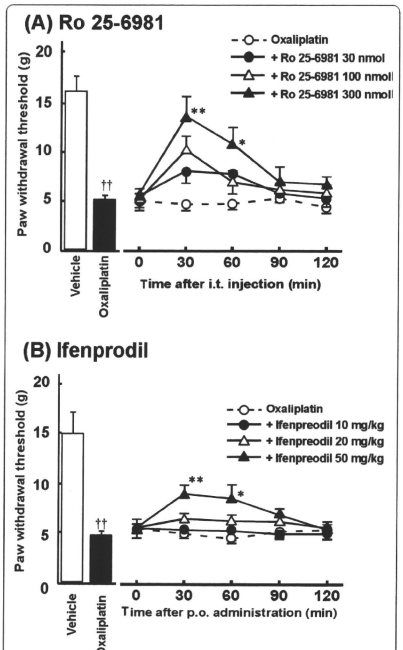

Figure 2 Effects of Ro 25-6981 (A) and ifenprodil (B) on oxaliplatin-induced mechanical allodynia in the von Frey test. Oxaliplatin (4 mg/kg) was administered i.p. twice a week for 4 weeks (Days 1, 2, 8, 9, 15, 16, 22 and 23). We confirmed the incidence of mechanical allodynia on Day 24. We carried out the drug evaluation on the next day. Ro 25-6981 (30-300 nmol/body) was administered intrathecally. Ifenprodil (10-50 mg/kg) was administered orally. The von Frey test was performed immediately before (0 min) and at 30, 60, 90 and 120 min after administration. Values are expressed as mean ± SEM of 6-7 animals. $^{\dagger\dagger}P < 0.01$ compared with vehicle, $^{*}P < 0.05$, $^{**}P < 0.01$ compared with oxaliplatin alone.

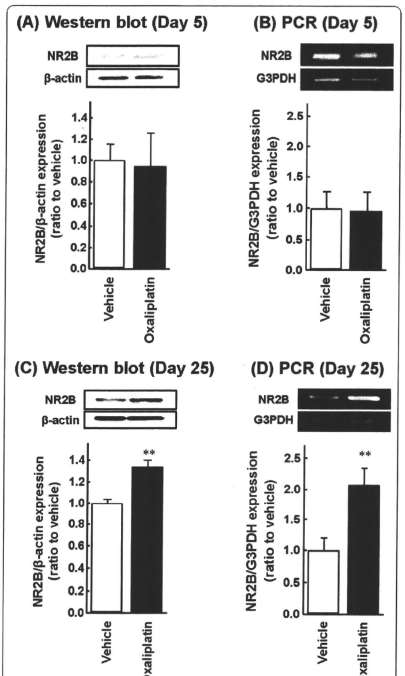

Figure 3 Changes in the NR2B protein (A, C) and mRNA (B, D) levels in the spinal cord in oxaliplatin-treated rats. Oxaliplatin (4 mg/kg) was administered i.p. twice a week for 1 (Days 1 and 2) or 4 weeks (Days 1, 2, 8, 9, 15, 16, 22 and 23). Western blot and PCR were performed to measure NR2B expression at the time points of Days 5 (A, B) and 25 (C, D) in the L4-6 spinal cord. Values are expressed as mean ± SEM of 6-9 animals. $^{**}P < 0.01$ compared with vehicle.

Changes of NR2B protein and mRNA in the spinal cord in oxaliplatin-treated rats

NR2B expression was examined by Western blot and polymerase chain reaction (PCR) analysis on homogenates of the spinal cord from rats. The results of Western blot and PCR showed that NR2B protein and mRNA levels in the spinal cord of oxaliplatin-treated rats significantly increased compared with that of vehicle-treated rats on Day 25 ($P < 0.01$, Figures 3C, D). On the other hand, oxaliplatin caused no change in NR2B protein and mRNA levels in the spinal cord on Day 5 (Figures 3A, B).

Effects of NOS inhibitors on oxaliplatin-induced mechanical allodynia

Acute administration of a non-selective NOS inhibitor L-NAME (300 μg, i.t.) completely reversed the reduction of paw withdrawal threshold by oxaliplatin at 30 min after administration ($P < 0.05$, Figure 4A). Similarly, acute administration of an nNOS inhibitor

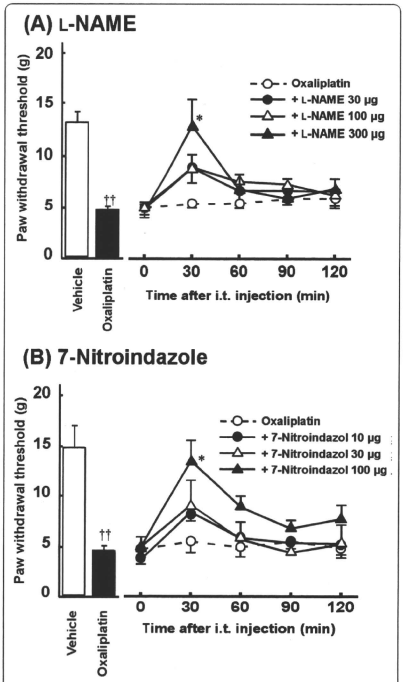

Figure 4 Effects of L-NAME (A) and 7-nitroindazole (B) on oxaliplatin-induced mechanical allodynia in the von Frey test. Oxaliplatin (4 mg/kg) was administered i.p. twice a week for 4 weeks (Days 1, 2, 8, 9, 15, 16, 22 and 23). We confirmed the incidence of mechanical allodynia on Day 24. We carried out the drug evaluation on the next day. L-NAME (30-300 μg/body) and 7-nitroindazole (10-100 μg/body) were administered intrathecally. The von Frey test was performed immediately before (0 min) and at 30, 60, 90 and 120 min after administration. Values are expressed as mean ± SEM of 6-10 animals. $^{\dagger\dagger}P < 0.01$ compared with vehicle, $^{*}P < 0.05$ compared with oxaliplatin alone.

7-nitroindazole (100 μg, i.t.) significantly inhibited the reduction of paw withdrawal threshold by oxaliplatin at 30 min after administration ($P < 0.05$, Figure 4B). These effects of L-NAME and 7-nitroindazole disappeared by 120 min after administration. In addition, L-NAME (300 μg, i.t.) and 7-nitroindazol (100 μg, i.t.) had no effect on the paw withdrawal thresholds in intact rats (data not shown).

Change of NOS activity in the spinal cord of oxaliplatin-treated rats

To evaluate change of NOS activity in oxaliplatin-induced mechanical allodynia, we carried out the NADPH diaphorase staining, a histochemical marker for NOS, in rat spinal cord sections. The results of NADPH diaphorase histochemistry revealed that the intensity of NADPH diaphorase staining (blue staining) obviously increased in the superficial layer of spinal dorsal horn in oxaliplatin-treated rats on Day 25 (Figure 5). Moreover, this increased intensity was reversed by intrathecal injection of Ro 25-6981 (300 nmol).

Discussion

In this study, NMDA receptor antagonists completely reverse the oxaliplatin-induced mechanical allodynia when administered after the development of neuropathy. Similarly, selective NR2B antagonists significantly inhibited the oxaliplatin-induced mechanical allodynia. Moreover, the expression of NR2B protein and mRNA in the spinal cord increased in the oxaliplatin-treated rats on Day 25 (late phase) but not on Day 5 (early phase). Oxaliplatin (4 mg/kg, i.p., twice a week) induces cold hyperalgesia in the early phase and mechanical allodynia in the late phase [14]. These findings suggest that the up-regulation of spinal NR2B-containing NMDA receptors is involved in the incidence of mechanical allodynia by repeated administration of oxaliplatin.

To investigate whether spinal cord NOS as the downstream target of NMDA receptor contributes to the incidence of mechanical allodynia, we examined the effects of NOS inhibitors on the oxaliplatin-induced mechanical allodynia. Intrathecal injection of L-NAME, a non-selective NOS inhibitor, and 7-nitroindazole, a selective nNOS inhibitor, inhibited the pain behavior, suggesting that NOS especially nNOS is involved in the oxaliplatin-

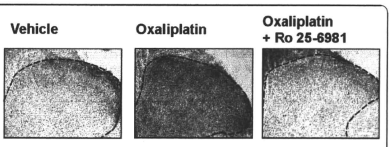

Figure 5 Typical photomicrographs representing NOS histochemistry staining of neurons in the spinal cord. Oxaliplatin (4 mg/kg) was administered i.p. twice a week for 4 weeks (Days 1, 2, 8, 9, 15, 16, 22 and 23). On Day 25, Ro 25-6981 (300 nmol/body) was administered intrathecally, and the L4-6 spinal cord was removed 30 min after administration. NADPH diaphorase staining was performed to confirm NOS activity. The blue staining shows NADPH diaphorase staining. Spinal slices (30 μm) were prepared from rats treated with vehicle (A), oxaliplatin alone (B), and oxaliplatin and Ro 25-6981 (C). Scale bar, 200 μm.

induced mechanical allodynia. This is further supported by the finding that the intensity of NADPH diaphorase staining in the rat spinal dorsal horn was increased by repeated administration of oxaliplatin, and that this increased intensity was reversed by intrathecal injection of Ro25-6981, which attenuated the oxaliplatin-induced pain behavior. Marked increase of nNOS expression in the dorsal root ganglia (DRG) and spinal cord contributes to spinal sensory processing in CCI model [11]. More recent experiments with selective NOS inhibitors and in NOS-deficient mice revealed the nNOS to be the most important NO-producing enzyme in the spinal cord during the development and maintenance of neuropathic pain in SNL model [17]. In mice with neuropathic pain by transection of spinal nerve, an increase in nNOS activity is visualized in the superficial dorsal horn by NADPH diaphorase histochemistry [18]. Taken together, these findings suggest that NOS especially nNOS contributes to the incidence of oxaliplatin-induced mechanical allodynia.

Interestingly, our results show that both oxaliplatin-induced pain behavior and increase of NOS activity are reversed by intrathecal injection of Ro25-6981, a selective NR2B antagonist. Phosphorylation of NMDA receptor NR2B subunits increases nNOS activity in the superficial dorsal horn of mice with neuropathic pain [18]. The activation of NMDA receptor also induces glutamate release through NOS activity [19]. Thus, the NMDA receptor and NOS comprise a local circuit that amplifies the signal of pain transmission. If sustained production of these factors by repeated administration of oxaliplatin is required for maintenance of mechanical allodynia, and if their treatment-induced increase is likely to cause persistence of pain, blockade of this circuit by the NR2B antagonist would likely reduce pain excitatory neurotransmission in the spinal cord. All of these findings indicate that NR2B antagonists have analgesic effects on the oxaliplatin-induced mechanical allodynia at the spinal level.

Non-competitive NMDA receptor antagonists are used as analgesics in clinical practice, although undesirable side effects limit their utility [20]. In contrast, the restricted distribution of NR2B receptor makes them promising candidates as targets of side effect-free analgesic drugs [21]. Indeed, ifenprodil, traxoprodil (CP-101606) and Ro25-6981 are effective in inflammatory and/or neuropathic pain models in animals at doses that are not accompanied by motor effects [8,22]. In addition, ifenprodil has been used as analgesic adjuvant in clinical settings. In this study, our results showed that NMDA receptor antagonists, selective NR2B antagonists and NOS inhibitors at the effective dose had no effect on pain behavior in intact rats. Therefore, the ameliorative effects of these drugs were not attributable to non-specific sedative effects or a deficit of motor function, suggesting that the reduced pain behavior reflects a therapeutic effect on oxaliplatin-induced mechanical allodynia. Novel strategies involving NR2B antagonists may be a useful alternative or adjunct therapy for oxaliplatin-induced peripheral neuropathy.

Conclusion

Our results indicate that repeated administration of oxaliplatin induces NR2B and NOS up-regulation in the spinal cord. This up-regulation may contribute to the incidence of mechanical allodynia. Furthermore, NMDA receptor antagonists, selective NR2B antagonists and NOS inhibitors remarkably attenuated the oxaliplatin-induced pain behavior. In addition, the selective NR2B antagonist inhibited the increase of NOS activity in the spinal cord. These results suggest that activation of the NMDA-NOS pathway contributes to the incidence of mechanical allodynia induced by repeated administration of oxaliplatin.

Methods

Animals

Male Sprague-Dawley rats weighing 200-250 g (Kyudo Co., Saga, Japan) were used in the present study. Rats were housed in groups of four to five per cage, with lights on from 7:00 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 [23].

Production of neuropathy

Mechanical allodynia was induced according to the method described previously [24]. Oxaliplatin (Elplat®) was obtained from Yakult Co., Ltd. (Tokyo, Japan). Oxaliplatin was dissolved in 5% glucose solution. The vehicle-treated rats were injected with 5% glucose solution. Oxaliplatin (4 mg/kg) or vehicle (5% glucose) was injected i.p. in volumes of 1 mL/kg twice a week for 4 weeks.

von Frey test

The mechanical allodynia was assessed by von Frey test. Each rat was 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, UK) ranging 1-15 g bending force were applied to the midplantar skin of each hind paw with each application held for 6 s. The paw withdrawal threshold was determined by a modified up-down method [25].

Pharmacological studies

We confirmed the incidence of mechanical allodynia on Day 24. We carried out the drug evaluation on the next day. The von Frey test was performed immediately before (0 min) and at 30, 60, 90, and 120 min after administration. (+)-MK-801 maleate (Wako Pure Chemical Industries, Ltd., Osaka, Japan), memantine hydrochloride (Alexis Biochemicals, San Diego, CA, USA) and L-NAME (Sigma-Aldrich, Missouri, USA) were dissolved in saline and administered i.t. Ro 25-6981 (Sigma-Aldrich) and 7-nitroindazole were dissolved in 100% dimethyl sulfoxide (DMSO) and administered i.t. Ifenprodil tartrate (Wako Pure Chemical Industries, Ltd.) was suspended in 5% gum arabic solution and administered orally (p.o.). The doses of these drugs were chosen based on previous reports [8,26-28]. Behavioral test was performed blindly with respect to drug administration.

Western blotting

To investigate the functional changes in protein levels of NR2B, the L4-6 spinal cord was quickly removed on Days 5 and 25. The tissues were homogenized in a solubilization buffer containing 20 mM Tris-HCl (pH7.4, 2 mM EDTA, 0.5 mM EGTA, 10 mM NaF, 1 mM Na_3VO_4, 1 mM PMSF, 0.32 M Sucrose, 2 mg/ml aprotinine, 2 mg/ml leupeptin), and the homogenates were subjected to 6% SDS-PAGE, and proteins were transferred electrophoretically to PVDF membranes. The membranes were blocked in Tris-buffered saline Tween-20 (TBST) containing 5% BSA (Sigma-Aldrich) for an additional 1 h at room temperature with agitation. The membrane was incubated overnight at 4°C with rabbit polyclonal NR2B antibody (1:5000; Upstate Biotech, NY, USA) and then incubated for 1 h with anti-rabbit IgG horseradish peroxidase (1:5000; Jackson Immuno Research Laboratories, Inc., PA, USA). The immunoreactivity was detected using Enhanced Chemiluminescence (Perkin Elmer, Massachusetts, USA).

Reverse transcriptase-polymerase chain reaction (RT-PCR)

To investigate the functional changes in mRNA levels of NR2B, the L4-6 spinal cord was quickly removed on Days 5 and 25. mRNA was isolated using PolyATtract® System 1000 (Promega, Corp., Wisconsin, USA). cDNA was synthesized using PrimScript® 1st strand cDNA Synthesis Kit (TaKaRa Bio, Inc., Shiga, Japan). PCR was carried out with Gene Taq (Nippon Gene, Co., Ltd., Tokyo, Japan). The oligonucleotide primers for NR2B were designed based on the sequences described by Lau et al. [29]. The sequences of PCR primers were as follows: NR2B, 5'-TCC GTC TTT CTT ATG TGG ATA TGC-3' (sense), 5'-CCT CTA GGC GGA CAG ATT AAG G-3' (antisense); glyceraldehyde-3-phosphate dehydrogenase (G3PDH), 5'-YGC CTG CTT CAC CAC CTT-3' (sense), 5'-TGC MTC CTG CAC CAC CAA CT-3' (antisense) (Sigma-Aldrich). Reactions were run for 40 cycles with 95°C denaturing cycle (30 s), 63°C annealing cycle (1 min) and 72°C extension cycle (30 s) for NR2B or for 30 cycles with 94°C denaturing cycle (45 s), 53°C annealing cycle (45 s) and 72°C extension cycle (1.5 min) for G3PDH, respectively. The PCR products were subjected to electrophoresis on 2% agarose gel, and the DNA was visualized by staining with ethidium bromide under ultraviolet irradiation. Then, the intensities of PCR products were semi-quantified densitometrically by Alpha Imager 2200 (Cell Biosciences, Inc., California, USA).

NADPH diaphorase histochemistry

Animals were anaesthetized with pentobarbital (50 mg/kg) and perfused through the left cardiac ventricle with 50 mL physiological saline followed by a fixative containing 4% paraformaldehyde in 0.1 M sodium phosphate (pH 7.4). The L4-6 spinal cord was removed and immersed in the fixative for 4 h and then cryoprotected overnight in 30% (w/v) sucrose in 0.1 M phosphate-buffered saline (pH 7.4). Transverse frozen sections (30 μm) were cut on a cryostat. These sections were thaw-mounted on slides and NOS activity was determined using NADPH diaphorase histochemistry as described by Mabuchi et al. [30]. The incubation was performed for 1 h at 37°C in a reaction mixture containing 0.5 mg/mL β-NADPH, 0.2 mg/mL nitroblue tetrazolium and 0.25% Triton X-100 in 0.1 M phosphate-buffered saline (pH 7.4).

Statistical analyses

Values were expressed as mean ± SEM. Results were analyzed by Student's *t*-test or one-way analysis of variance (ANOVA) followed by the Tukey-Kramer post-hoc test to determine differences among the groups. A *P* value of less than 0.05 is considered as statistically significant.

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Authors' contributions

YM, NE, TK, TY, HI and RO are responsible for experimental design. YM and HS are responsible for performance of behavioral test. YM, HS, TK and SU are responsible for performance of Western blot, PCR and NADPH diaphorase staining. YM, NE, HS, TK 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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SURGERY TODAY

Review Article

The Effect of Traditional Japanese Medicine (Kampo) on Gastrointestinal Function

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Abstract
Traditional Japanese medicine (Kampo) is used to treat various disorders of the gastrointestinal tract in Japan, where it is fully integrated into the modern healthcare system. Recently, scientific research on herbal medicine in Japan has been reported in English journals. The objective of the current review is to introduce two traditional Japanese medicines and to provide evidenced-based information regarding their use. Daikenchuto, which consists of three different herbs, is the most frequently prescribed traditional Japanese medicine in Japan. Daikenchuto stimulates gastrointestinal motility though a neural reflex involving presynaptic cholinergic and 5-HT3 receptors. Daikenchuto improves postoperative bowel motility and postoperative ileus. Furthermore, it is reported to cause an increase in gastrointestinal hormones (motilin, vasoactive intestinal peptide, and calcitonin gene-related peptide) and intestinal blood flow. Rikkunshito, a traditional Japanese medicine consisting of eight herbs, is thought to stimulate gastrointestinal motility and ghrelin secretion. Rikkunshito is effective for improving the symptoms of functional dyspepsia, gastroesophageal reflux disease, and cisplatin-induced anorexia and vomiting. Traditional Japanese medicine has the potential to be used successfully in the treatment of gastrointestinal disorders. Details regarding the physiological and clinical effects of traditional Japanese medicine must be further examined in order to become more widely accepted in other countries.

Key words Traditional Japanese medicine · Kampo · Rikkunshito · Daikenchuto · Gastrointestinal function

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Recent data for this review were collected by Medline searches using the following keywords: "traditional Japanese medicine," "Japanese herbal medicine," "herbal medicine," and "kampo."

Introduction

Traditional Japanese medicine, which includes Kampo, acupuncture, and acupressure, has been used for 1500 years. The use of Kampo, or herbal medicine, is based on extensive experience with herbal combinations accumulated in East Asia since ancient times. Kampo is intended to boost the body's own healing power and help restore its natural balance. Traditional Japanese medicine is widely practiced in Japan, where it is fully integrated into the modern health-care system.

The National Center for Complementary and Alternative Medicine, established at the National Institutes of Health in the United States in October 1998, recategorized traditional medicine as "complementary and alternative medicine" following the increased interest in non-Western medicine.[1] Recently, scientific research on herbal medicine in Japan has been reported in English-language journals, and several rigorous clinical and basic research studies have confirmed the effects of traditional Japanese medicine. As a result, the United States Food and Drug Administration began to pay more attention to traditional medicine, especially traditional Japanese medicine, noting its exceptionally high quality and standardized ingredients. The objective of this review is to introduce two traditional Japanese medicines and to provide evidenced-based information regarding their effects and use.

Gastrointestinal Function (Motility)

The gastrointestinal tract has distinct contractile patterns. Gastrointestinal motility is clearly divided into two phases: the interdigestive state and the postprandial state. During the interdigestive state, the gastrointestinal tract exhibits a characteristic motor pattern called interdigestive migration motor contraction (IMC),[2] which consists of four phases, with a combined duration

of about 100 min. Phase I is a quiescent period; phase II is intermittent contractions; phase III involves intense, rhythmic contractions that begin in the gastric body and propagate to the small intestine; and phase IV consists of intermittent contractions following phase III. It is thought that the physiological role of the IMC is to expel undigested food particles, mucus, and sloughed epithelial cells from the stomach and/or small intestine. Motilin is an important factor in the initiation of the regular occurrence of IMC of the stomach.[3] A meal interrupts this well-defined pattern and triggers phasic contractions of variable intensity that occur almost continuously. However, the gastric fundus relaxes after a meal, and gastric accommodation in response to gastric distension is mediated by the stimulation of gastric mechanoreceptors.

Daikenchuto

Daikenchuto (DKT) is the most frequently prescribed traditional medicine in Japan. Daikenchuto consists of three different herbs: 50% dried ginger rhizome, 30% ginseng root, and 20% zanthoxylum fruit. Daikenchuto has been used for the treatment of abdominal obstruction, including bowel obstruction, and a feeling of coldness in the abdomen. Since the first report on the use of DKT for intestinal obstruction was published in 1987, DKT has often been used in the treatment of postoperative ileus after gastrointestinal surgery. Daikenchuto, which has been on the Japanese market for several decades, has been shown to be effective for bowel disorders and to cause very few side effects.[4] Table 1 summarizes the effects of DKT on gastrointestinal function.

Effect of DKT on Gastrointestinal Motor Activity

Daikenchuto has been found to significantly increase gastrointestinal motility. Intragastric DKT induced phasic contractions in the pylorus, duodenum, jejunum, and ileum in conscious dogs (Fig. 1A). A burst of contractions of an intensity similar to that of IMC occurred at the stomach, and these contractions synchronistically or rapidly migrated aborally. Shibata et al. reported that DKT-induced contractions in the gastric antrum were found to be caused mainly by the dried ginger rhizome, whereas those in the duodenum and jejunum were caused mainly by the zanthoxylum fruit.[5] Concerning the mechanism responsible for these contractile effects, DKT in the gastric lumen stimulates enteric neurons to release acetylcholine (ACh) as the effective neurotransmitter in the gastric antrum and duodenum through a neural reflex involving presynaptic cholinergic and the 5-hydroxytryptamine (serotonin) 5-HT3 receptors.[5] However, it was reported that the contractions induced by DKT were not suppressed by a 5-HT3 receptor antagonist in isolated guinea-pig ileum.[6]

Kawasaki et al. reported that the prokinetic effects of DKT were evident at the stomach, jejunum, and proximal colon only during the fasting state.[7] However, with intracolonic administration, a contraction similar to the giant migrating contraction was induced during both the fasting and fed states, and defecation occurred. The reason for this difference could be that the colon is continuously filled with fecal clumps, and thus is not greatly affected by changes in the internal environment resulting from dietary intake, unlike the upper gastrointestinal tract, which empties completely during fasting. In a human study, DKT stimulated colonic motility immediately after administration into the ascending colon.[8] Daikenchuto in the colonic lumen stimulates enteric neurons, and intestinal contraction may be induced by the local nervous system in the colonic wall.

There have been several interesting reports concerning the pharmacological action of DKT. In experiments on isolated intestines, studies showed that DKT induced contraction and ACh release in the guinea pig distal colon[9] and increased contraction in the rabbit jejunum.[10] In these reports, it was concluded that the zanthoxylum fruit acts as a stimulant in DKT-induced intestinal motility. Furthermore, DKT-induced contraction was accompanied by autonomous contraction at a concentration of more than 3×10^{-4} g/ml in a dose-dependent manner in the isolated guinea-pig ileum.[6] The contraction induced by DKT was inhibited by atropine, tetrodotoxin, and norepinephrine, but not by the ganglion blocker, hexamethonium. This effect was partially suppressed in the presence of high concentrations of ICS205–930, a 5-HT4 receptor antagonist. In addition, DKT showed ACh-releasing action in the smooth muscle tissues of the ileum. These results suggest that the contractile response induced by DKT is partially mediated by the ACh released from the cholinergic nerve endings, and that 5-HT4 receptors are involved in the effect of DKT.[6] Tachykinins from sensory neurons other than ACh released from the cholinergic neuron are also involved in the contraction induced by zanthoxylum fruit and in atropine-resistant contraction induced by DKT.[11]

Effect of DKT on Gastrointestinal Hormones

Daikenchuto caused significant increases in the plasma levels of motilin,[12,13] vasoactive intestinal peptide (VIP),[14] 5-HT,[14] calcitonin gene-related peptide (CGRP), and substance P.[13] Sato et al. reported that DKT may stimulate motilin and VIP release via M1 muscarinic receptors, and that CGRP and substance P

Table 1. Effects of Daikenchuto and Rikkunshito on gastrointestinal function

Daikenchuto	Stimulation of gastrointestinal motility Improvement of post-operative bowel motility Increase in intestinal and colonic blood flow Stimulation of motilin, VIP, CGRP, and substance P release Improvement of postoperative ileus Improvement of morphine-induced disorder of gastrointestinal transit Prevention of bacterial translocation Improvement of stasis after total gastrectomy
Rikkunshito	Stimulation of gastrointestinal motility Facilitation of gastric emptying Improvement of gastroesophageal reflux Improvement of FD symptoms Stimulation of ghrelin secretion Improvement of cisplatin-induced anorexia and vomiting

VIP, vasoactive intestinal peptide; CGRP, calcitonin gene-related peptide; FD, functional dyspepsia

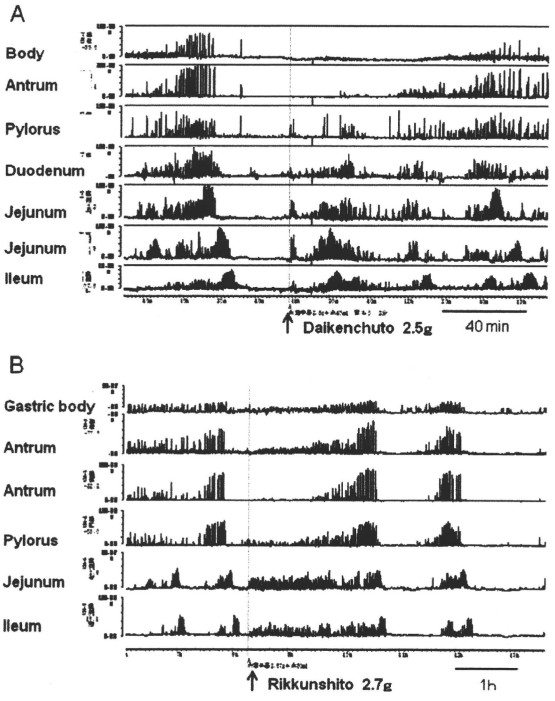

Fig. 1. A Gastrointestinal motor activity measured using a strain-gauge force transducer in conscious dogs. During the interdigestive state the gastrointestinal tract exhibited a characteristic motor pattern called interdigestive migration motor contraction, which began in the stomach and progressed to the small intestine. Intragastric administration of Daikenchuto induced phasic contractions in the pylorus, duodenum, and jejunum. **B** During the interdigestive state, the intragastric administration of Rikkunshito induced a burst of contractions similar to postprandial contractions

release may be related, in part, to M1 muscarinic receptors.[13] In fact, muscarinic receptors are present on the membrane of motilin cells, and ACh is a major regulator of motilin release by acting on M1 or M3 muscarinic receptors.[3,15] However, to conclusively prove the effect of DKT on gastrointestinal hormones, more detailed studies must be conducted.

Effect of DKT on Intestinal Blood Flow

Murata et al. reported that DKT produced an increase in intestinal blood flow in rats.[16] However, DKT did not affect the mean arterial pressure, even when administered at the highest dose, which suggests that the increase in intestinal blood flow by DKT results from a local vasodilator effect in intestinal vessels rather than secondary changes in systemic blood pressure. In contrast, cisapride, a prokinetic agent that has been used in the treatment of intestinal obstruction, failed to increase intestinal blood flow.[16] Concerning colonic blood flow, Kono et al. reported that intracolonic administration of DKT increased colonic vascular conductance in rats.[17] The CGRP receptor antagonist, CGRP(8–37), completely abolished DKT-induced hyperemia, whereas the VIP receptor antagonist, [4-Cl-DPhe6,Leu17]-VIP, and an SP receptor antagonist did not attenuate the hyperemic response.[17]

Calcitonin gene-related peptide is a powerful vasoactive substance that is released from the sensory afferent nerve endings. Calcitonin gene-related peptide increases gastric mucosal blood flow as a gastroprotective factor, which suggests that the hyperemic response to DKT results primarily from the upregulation of CGRP release and/or the upregulation of the CGRP receptor.[17] Furthermore, it has been reported that DKT can activate CGRP secretion from mucosal sensory nerve endings and adrenomedullin release from mucosal epithelial cells.[4] In our clinic, patients have reported feeling a warm sensation in the abdomen when given DKT.

Effect of DKT on Postoperative Ileus

In Japan, DKT has been used to treat adhesive bowel obstruction after surgery.[18] Postoperative ileus remains the most common complication after abdominal surgery, and delayed return of gastrointestinal function and resumption of oral intake are major causes for prolonged hospitalization. Daikenchuto shortens the time period until the first defecation and the duration of hospital stay of patients with adhesive bowel obstruction.[18] The pathogenesis of postoperative ileus is multifactorial, and is thought to be mainly a neural reflex associated with inflammatory responses, intestinal replacement, and dryness.[19] Uemura et al. reported that the incision of the abdominal wall causes postoperative ileus, and that additional intestinal manipulation further promotes postoperative ileus.[20] Treatment of DKT has been shown to improve delayed intestinal transit and intestinal adhesion after laparotomy in rats.[21] Stimulation of gastrointestinal contractions and anti-inflammatory action may be responsible for the improvement of postoperative ileus by DKT.[21] Furthermore, Fukuda et al. reported that DKT improved the postoperative hypoperistalsis via cholinergic nerves and 5-HT3 and 5-HT4 receptors.[22] It has been suggested that the stimulatory effect of DKT on gastrointestinal motility is mediated via serotonergic pathways as well as cholinergic pathways in vivo. The stimulatory effect of DKT is abolished in rats treated with atropine and vagotomy.[22] These findings suggest that an intact vagal cholinergic pathway is needed to promote the prokinetic effect of DKT in postoperative ileus.

Postoperative ileus is often exacerbated by opiate analgesic use during and after surgery. Morphine slows gastrointestinal transit by inhibiting the relaxation of circular muscle and the contraction of longitudinal muscles.[23] Daikenchuto is thought to have a restorative effect on delayed gastrointestinal transit induced by intestinal manipulation and morphine administration.[22,24]

Although a total gastrectomy is widely performed for the treatment of gastric carcinoma, there is no general agreement about ideal reconstruction. To provide reservoir function and to improve nutritional conditions, many types of jejunal pouch reconstruction have been described and evaluated.[2] Some patients, however, remained symptomatic after pouch addition, and most had abnormal or disturbed intestinal motility.[25] Endo et al. reported that DKT increased pouch contractions, accelerated the emptying of liquid and solid materials from the pouch, and decreased postoperative stasis-related symptoms.[26]

Rikkunshito

Rikkunshito (RKT; 7.5 g), a traditional herbal medicine, is a mixture of dried Atractylodis lanceae rhizoma (0.75 g), ginseng radix (0.74 g), pinelliae tuber (0.74 g), hoelen (0.74 g), Zizyphi fructus (0.37 g), Aurantii nobilis pericarpium (0.37 g), Glycyrrhizae radix (0.20 g), Zingiberis rhizome (0.10 g), and spray-dried aqueous extract (4.0 g). This is a fixed-ratio formulation of eight medicinal herbs and roots, and the quality and volume of ingredients are uniform. Rikkunshito is widely prescribed in Japan for patients with a variety of gastrointestinal symptoms, including anorexia, nausea, and vomiting.[27] The effects of RKT on gastrointestinal function are summarized in Table 1.

Effect of RKT on Gastrointestinal Motor Activity and Gastric Emptying

Data on the effectiveness of RKT on gastrointestinal motor activity are limited in the English-language literature. The results of our preliminary study showed that RKT stimulates gastrointestinal motility (Fig. 1B). Intragastric RKT induced a burst of contractions similar to the postprandial contractions at the antrum, pylorus, jejunum, and ileum, and did not inhibit the occurrence of the next phase III. Rikkunshito is a mixture of several crude extracts, and is reported to have dual actions on the stomach: relaxation of the proximal stomach and increased contractions of the distal stomach.[28,29] However, RKT has not yet been evaluated regarding its influence on gastrointestinal motor activity.

In Japan, RKT is widely prescribed for patients with chronic hypofunction of the gastrointestinal tract, including gastric flatulence, anorexia, nausea, and vomiting. Tatsuta and Iishi reported that oral administration of RKT to patients significantly reduced chronic dyspepsia and produced significantly better gastric emptying than did the administration of a placebo.[28] In addition, Kido et al. reported that RKT was shown to ameliorate the delay in gastric emptying induced by N^G-nitro-L-arginine, and that its main active ingredient for improving motility disorders of the stomach was likely hesperidin, identified from its methanol fraction by highly porous polymer chromatography.[30]

The mechanism of action responsible for the effects of RKT on gastric emptying may be related to the nitric oxide pathway. L-Arginine (a substrate for nitric oxide production) is contained in RKT at a concentration of 0.9% (4.5 mg/500 mg of RKT). Tominaga et al. showed that RKT could improve the 5-HT-induced delay in gastric emptying in rats, the mechanism of which appears to involve the antagonistic action of the 5-HT3 receptor pathway.[31] Several studies have shown that RKT has clinical efficacy for functional dyspepsia (FD) via the improvement of gastrointestinal motility disorders. Rikkunshito is thought to improve FD by reversing the existing impaired adaptive relaxation, leading to an improvement in delayed gastric emptying.[29,30,32] Functional dyspepsia patients have been shown to exhibit impaired reservoir function, including gastric adaptive relaxation.[33] The results of an in vitro study showed that RKT evoked relaxation with two components, an initial fast component, followed by a second component with a slower time course, in isolated rat fundus smooth muscle.[34] These results suggest that RKT promotes gastric adaptive relaxation and relieves symptoms in patients with FD. Furthermore, it has been reported that RKT relieves stasis-related symptoms of patients following pylorus-preserving gastrectomy and accelerates emptying of solids from the remnant stomach.[35]

Effect of RKT on Gastroesophageal Reflux

In recent years, RKT has been used to treat symptoms of gastroesophageal reflux disease (GERD), and reports of its efficacy have been published.[36,37] In children with symptomatic GERD, RKT relieved symptoms and reduced distal esophageal acid exposure through improved esophageal acid clearance.[36] Rikkunshito did not change the number of acid reflux events, but did reduce the esophageal acid clearance time. However, the mechanism underlying the improved esophageal clearance capacity with RKT remains unknown.

Effect of RKT on a Gastrointestinal Hormone (Ghrelin)

Ghrelin, an endogenous ligand for the growth hormone secretagogue receptor, was originally isolated from human and rat stomachs.[38] Ghrelin is known to have an intense appetite-enhancing effect, in addition to its growth hormone secretion-promoting effect, and it also stimulates gastric motility and gastric acid secretion.[39,40] However, Ohno et al. reported that ghrelin did not stimulate gastrointestinal motility in dogs.[41] Takeda et al. reported that the cisplatin-induced decreases of plasma ghrelin levels were suppressed by RKT in rats.[42] In healthy human volunteers, RKT increased the plasma acylated ghrelin level, but the level of plasma deacylated ghrelin did not change.[43] This effect was maintained for at least 4 weeks after the end of RKT administration. Furthermore, in mice the mRNA expression level of ghrelin in gastric tissue was upregulated after 2 weeks' administration of RKT.[43] RKT stimulates endogenous ghrelin secretion from the stomach via 5-HT2B and 5-HT2C receptor antagonism.[42,44] The 5-HT system in the gastrointestinal tract has an important role in the regulation of gastrointestinal motor activities. Selective serotonin reuptake inhibitors (SSRIs) changed the phase III-like contractions to fed-like motor activities.[44] Fujitsuka et al. reported that the fed-like motor activities induced by the intravenous administration of SSRIs were replaced by fasted motor activities after RKT administration. This motor effect of RKT is blocked by the intravenous administration of a growth hormone secretagogue receptor antagonist (ghrelin receptor antagonist), which suggests that RKT stimulates ghrelin secretion.

Takeda et al. reported that the plasma level of ghrelin in fasted aged mice was significantly decreased compared with that in young mice.[45] In aged mice, RKT increased the reactivity of ghrelin and ameliorated aging-associated anorexia.[45] The results of this study suggest the possibility of using RKT to treat aging-associated anorexia.

Effect of RKT on Chemotherapy-Induced Nausea and Appetite Loss

Cisplatin is widely used in chemotherapy to treat various types of cancer. However, it has gastrointestinal side effects such as nausea, vomiting, and anorexia, which markedly affect the quality of life of patients. Cisplatin-treated rats show acute decreases in the concentration of circulating ghrelin and appetite loss.[42] In 2006, Tomono et al. reported that RKT with antiemetics is more effective than antiemetics alone against anorexia during chemotherapy in patients with advanced breast cancer.[46] This finding suggests that RKT may be a good antiemetic agent. One possible mechanism by which RKT exerts this effect is via the enhancement of the circulating ghrelin concentration. Cisplatin-induced decreases in the plasma acylated ghrelin level and food intake are mediated by 5-HT2B/2C receptors and suppressed by flavonoids in RKT.[42] In addition, Yakabi et al. demonstrated that RKT and the 5-HT2C receptor antagonist may improve cisplatin-induced anorexia by inhibiting decreased hypothalamic growth hormone secretagogue receptor 1a (GHS-R1a) signal transduction.[47] Hence, RKT may be an orexigenic preparation that prevents cisplatin-induced anorexia and vomiting.

Conclusion

This review of traditional Japanese medicine illustrates the effect of two major herbal preparations on gastrointestinal function. Traditional Japanese medicine has the potential to successfully treat gastrointestinal disorders. However, further examinations of the physiological and clinical effects of traditional Japanese medicine through rigorous scientific research will be necessary before it can become more widely accepted, especially in the Western world.

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