under their national health insurance since 1986. In addition, DKT has been on the market in Japan for several decades and is associated with very few side effects.[43]

In summary, our findings indicate that DKT attenuates mucosal damage, colonic inflammatory adhesions, systemic inflammation, and inhibited mucosal proinflammatory cytokines, including TNFα and IFNγ, in a CD mouse model via up-regulation of endogenous ADM in the IE. Endogenous ADM in epithelial cells may be a unique therapeutic target for CD morbidity.

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Original Article

Efficacy of Goshajinkigan for Peripheral Neurotoxicity of Oxaliplatin in Patients with Advanced or Recurrent Colorectal Cancer

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Peripheral neurotoxicity is the major limiting factor for oxaliplatin therapy. Goshajinkigan (GJG), a traditional Japanese herbal medicine, was recently shown to be effective in protecting against the neurotoxicity of taxanes in Japan. We retrospectively investigated the effect of GJG on peripheral neurotoxicity associated with oxaliplatin therapy. Ninety patients with metastatic colorectal cancer that received FOLFOX4 or modified FOLFOX6 therapy were assigned to receive one of the following adjuncts: oral GJG at 7.5 g day^{-1} (Group A, $n = 11$), intravenous supplementation of calcium gluconate and magnesium sulfate (1 g each before and after FOLFOX) (Group B, $n = 14$), combined GJG and calcium gluconate and magnesium sulfate therapies (Group C, $n = 21$), or no concomitant therapy (Group D, $n = 44$). The incidence of peripheral neurotoxicity was investigated when the cumulative dose of oxaliplatin exceeded 500 mg m^{-2}. When the cumulative dose of oxaliplatin exceeded 500 mg m^{-2}, the incidence of neuropathy (all grades) in Groups A–D was 50.0%, 100%, 78.9%, and 91.7%, respectively. It was lowest in the group that received GJG alone. Concomitant administration of GJG reduced the neurotoxicity of oxaliplatin in patients that received chemotherapy for colorectal cancer.

1. Introduction

In recent years, the standard chemotherapy for advanced/recurrent colorectal cancer is a continuous intravenous infusion of 5-fluorouracil (5-FU) combined with either oxaliplatin (FOLFOX, FOLFOX4 or modified FOLFOX6) or irinotecan (FOLFIRI) [1–3]. Acute and persistent peripheral neuropathy is the characteristic of oxaliplatin therapy [4], and the oxaliplatin dose must be limited to avoid toxicity. The prevalence of peripheral neurotoxicity increases with the total accumulated dose of oxaliplatin, and often interferes with the continuation of FOLFOX therapy [5]. Gamelin et al. [6, 7] reported that administration of calcium gluconate and magnesium sulfate (Ca/Mg) before and after oxaliplatin therapy could alleviate peripheral neurotoxicity. Other similar treatments have been described, including carbamazepine [8–10] or glutathione [11], but an effective remedy for peripheral neurotoxicity related to oxaliplatin therapy has not yet been established.

Goshajinkigan (GJG) is an extracted traditional Japanese herbal medicine (Kampo) that is mainly used for the improvement of symptoms like numbness, cold sensation and limb pain associated with diabetic neuropathy [12–15]. Moreover, Mamiya et al. [16] and Shindo et al. [17] recently reported that peripheral neurotoxicity due to oxaliplatin was relieved by administration of GJG in patients with advanced colorectal cancer that were receiving FOLFOX therapy.

We conducted the present retrospective study to compare the efficacy of GJG with that of Ca/Mg for alleviation of peripheral neurotoxicity in patients with advanced or recurrent colorectal cancer that received either FOLFOX4 therapy or modified FOLFOX6 (mFOLFOX6) therapy at our hospital and affiliated institutions in Japan.

2. Patients and Methods

2.1. Patients. This retrospective analysis included 90 patients with advanced or recurrent colorectal cancer that had received either FOLFOX4 or mFOLFOX6 therapy from August 2005 to January 2008 at our hospital and five affiliated institutions. Patients were classified into the following four groups: chemotherapy + GJG, chemotherapy + Ca/Mg, chemotherapy + GJG + Ca/Mg and chemotherapy alone. Full ethical approval for this study has been obtained from all of each responsible Ethics Committees in each hospital according to Japanese Ministry of Health, Labour and Welfare guidelines. All patients provided written informed consent. All records will be kept confidential and the patient's; name will not be released at any time.

2.2. Chemotherapy. On Day 1, patients treated with FOLFOX4 received a 2-h intravenous infusion of oxaliplatin ($85\,mg\,m^{-2}$) combined with levofolinate (1-LV, $100\,mg\,m^{-2}$), followed by a bolus injection of 5-FU ($400\,mg\,m^{-2}$), and then continuous infusion of 5-FU ($600\,mg\,m^{-2}$) for 22 h. On Day 2, 1-LV ($100\,mg\,m^{-2}$) was administered in a 2-h intravenous infusion, and then a bolus of 5-FU was administered ($400\,mg\,m^{-2}$), followed by a 22-h continuous 5-FU infusion ($600\,mg\,m^{-2}$). This regimen comprised one course of therapy and was repeated once every 2 weeks.

On Day 1, patients treated with mFOLFOX6 therapy received a 2-h intravenous infusion of oxaliplatin ($85\,mg\,m^{-2}$) combined with 1-LV ($100\,mg\,m^{-2}$), followed by a rapid intravenous infusion of 5-FU ($400\,mg\,m^{-2}$), and then a 46-h continuous infusion of 5-FU ($2400\,mg\,m^{-2}$). This regimen comprised one course of therapy and was repeated once every 2 weeks.

GJG ($7.5\,mg\,day^{-1}$ divided into 2-3 doses) was administered during FOLFOX therapy, given orally before meals or between meals on a daily basis. Ca and Mg (1 g each) were administered before and after FOLFOX therapy by intravenous infusion.

2.3. Endpoints and Evaluation. Each group was evaluated to determine the total dose of oxaliplatin, the median and mean numbers of courses, the incidence of each grade of peripheral neuropathy, the incidence of peripheral neuropathy when the total dose of oxaliplatin exceeded $500\,mg\,m^{-2}$, the total dose of oxaliplatin at which 50% of patients showed peripheral neuropathy and the time to treatment failure (TTF). Peripheral neuropathy evaluations were based on the Neurotoxicity Criteria of DEBIOPHARM (DEBNTC) [18]. The assessment of the occurrence of peripheral neuropathy in relation to the total dose of oxaliplatin, and the TTF comparisons were based on Kaplan-Meier analyses. The attending physicians assessed the anti-tumor effect of chemotherapy with the new Guidelines for Evaluation of the Response to Treatment in Solid Tumors (RECIST) [19]. In this retrospective study, Groups A–C were compared with Group D (no adjunct treatment) with the log-rank test to identify differences in the incidence of peripheral neuropathy and TTF. Differences observed among groups in the incidence of peripheral neuropathy at a total oxaliplatin dose $>500\,mg\,m^{-2}$ and differences in the anti-tumor activity were assessed with the chi-square test.

3. Results

3.1. Patient Characteristics. There were an unequal number of patients in each group (Table 1). Group D (no concomitant therapy) was the largest group and Group A (GJG alone) was the smallest group. There were no between-group differences of sex (P-values are for Groups A ($P = .890$), B ($P = .223$) and C ($P = .745$) versus Group D by the χ^2-test) and age (P-values are for Groups A ($P = .954$), B ($P = .470$), and C ($P = .790$) versus Group D by the t-test). The performance status (PS) was evaluated with The Eastern Cooperative Oncology Group criteria. A higher percentage of Group A patients had a PS = 1 compared with the other groups. None of the patients in Groups A or B had a PS = 2, but there were no significant differences in PS between the groups (P-values are for Groups A ($P = .373$), B ($P = .316$) and C ($P = .702$) versus Group D by the χ^2-test). There were no differences in the locations of the primary and metastatic tumors (P-values are for Groups A ($P = .498$), B ($P = .431$) and C ($P = .993$) versus Group D by the χ^2-test).

3.2. FOLFOX Therapy. The chemotherapy regimen most commonly administered in Group A was mFOLFOX6, and all of the patients in Groups B and C received mFOLFOX6 therapy. Only Group D had a relatively large number of patients that received FOLFOX4 therapy. Table 2 shows the median and mean total doses of oxaliplatin for each group. Both the median and mean doses were highest in Group A, followed by Groups C, D and B in descending order. Compared with Group D, Group B had a smaller percentage of patients ($P = .004$ by the χ^2-test.) that received a total dose of oxaliplatin exceeding $500\,mg\,m^{-2}$, but there was no difference between the other two groups ($P = .466$ and .366 by the χ^2-test.). Groups A, C and D had similar medians and mean numbers of courses, but Group B had the lowest number of courses (Table 2).

3.3. Peripheral Neuropathy. Kaplan-Meier analyses showed that peripheral neuropathy of Grade 1 or worse (Figure 1) and Grade 2 or worse (Figure 2) occurred less frequently in Group A compared with the other groups; the difference was most marked for neuropathy of Grade 1 or worse. Peripheral neuropathy of Grade 3 (Figure 3) did not occur in either Groups A or C, the two groups that received GJG therapy.

The incidence of peripheral neuropathy at a total oxaliplatin dose $>500\,mg\,m^{-2}$ was lower in the two groups given GJG (Group A and Group C; Table 3). In Group A, there were no cases of Grades 2 or 3 peripheral neuropathy. In Group

Table 1: Patient characteristics for the four groups.

	Group A ($n = 11$)	Group B ($n = 14$)	Group C ($n = 21$)	Group D ($n = 44$)	Total ($n = 90$)
Sex					
Male	7	6	12	27	52
Female	4	8	9	17	38
Age, median (range)	62 (47–78)	61.5 (54–75)	63 (36–82)	64 (43–87)	63 (36–87)
Body weight, median (range)	59 (41–76)	60 (40–75)	58 (38–77)	59 (39–76)	59 (38–77)
PS					
0	7	13	15	38	73
1	4	1	4	3	12
2	0	0	2	3	5
Primary tumor					
Colon	4	5	10	21	40
Rectum	7	9	11	23	50
Metastasis					
Liver	9	9	12	28	58
Lung	3	5	4	18	30
Lymph nodes	0	2	1	1	4
Other	2	4	7	8	21

Group A, GJG; Group B, Ca/Mg; Group C, GJG + Ca/Mg; Group D, no therapy.
GJG, goshajinkigan; Ca, calcium gluconate; Mg, magnesium sulfate; PS, performance status.

Table 2: Details of FOLFOX therapy.

	Group A ($n = 11$)	Group B ($n = 14$)	Group C ($n = 21$)	Group D ($n = 44$)	Total ($n = 90$)
FOLFOX					
FOLFOX4	4	0	0	33	37
mFOLFOX6	7	14	21	11	53
Cumulative oxaliplatin Dose ($mg\,m^{-2}$)					
Median	807.5	500.0	750.0	680.0	680.0
Mean	726.3	534.3	686.7	625.0	632.3
Range	300–850	170–850	180–850	235–850	170–850
Total oxaliplatin dose $\geq 500\,mg\,m^{-2}$	90.9%	42.9%	90.5%	81.8%	78.9%
Percentage of patients in group	($n = 10$)	($n = 6$)	($n = 19$)	($n = 36$)	($n = 71$)
No. of courses					
Median	10.0	6.0	10.0	8.0	8.5
Mean	8.9	6.8	8.8	7.9	8.0
Range	4–10	2–10	3–10	3–10	2–10

Group A, GJG; Group B, Ca/Mg; Group C, GJG + Ca/Mg; Group D, no therapy.
GJG, goshajinkigan; Ca, calcium gluconate; Mg, magnesium sulfate.

B (Ca/Mg alone), the overall incidence of neuropathy was comparable to that in Group D (no concomitant therapy), but Group B had a higher rate of Grade 3 peripheral neuropathy (Table 3).

The total dose of oxaliplatin at which 50% of the patients developed peripheral neuropathy was $765\,mg\,m^{-2}$ in Group A for Grade 1 or worse neuropathy (Table 4). In Group A, 50% level was not reached for Grade 2 or worse neuropathy; this suggested that a higher oxaliplatin dose could be administered to Group A when compared to the other groups before peripheral neurotoxicity occurred.

Table 3: Frequency of peripheral neuropathy at a total oxaliplatin dose of 500 mg m^{-2}.

	Group A, (n = 10)		Group B, (n = 6)		Group C, (n = 19)		Group D (n = 36)
	Percentage	P-value	Percentage	P-value	Percentage	P-value	
All grades	50.0	.002	100	.463	78.9	.178	91.7
Grade 2	0	.130	16.7	.873	5.3	.156	19.4
Grade 3	0	.345	33.3	.080	0	.196	8.3

Group A, GJG; Group B, Ca/Mg; Group C, GJG + Ca/Mg; Group D, no therapy.
P-values are for Groups A, B and C versus. Group D by the χ^2-test.
GJG, goshajinkigan; Ca, calcium gluconate; Mg, magnesium sulfate.
n: numbers are patients received over 500 mg m^{-2} dose of total oxaliplatin.

Table 4: Total dose of oxaliplatin at which 50% of patients developed neuropathy.

	Group A (n = 11)	Group B (n = 14)	Group C (n = 21)	Group D (n = 44)
Grade ≥1	765	255	340	255
Grade ≥2	Not reached	510	765	670
Grade 3	—	850	—	Not reached

Total oxaliplatin doses are shown in mg/m^2.
Grade ≥1, Grade 1 or worse neuropathy; Grade ≥2, Grade 2 or worse neuropathy;
Group A, GJG; Group B, Ca/Mg; Group C, GJG + Ca/Mg; Group D, no therapy.
GJG, goshajinkigan; Ca, calcium gluconate; Mg, magnesium sulfate.

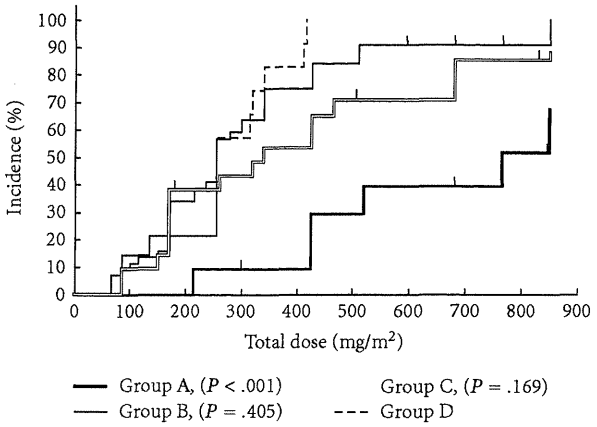

Figure 1: Kaplan-Meier analysis of Grade 1 or worse peripheral neuropathy in relation to the total dose of oxaliplatin. Group A, GJG; Group B, calcium gluconate (Ca) and magnesium sulfate (Mg); Group C, GJG + Ca/Mg; Group D, no therapy. P-values are for comparison to Group D with the log-rank test.

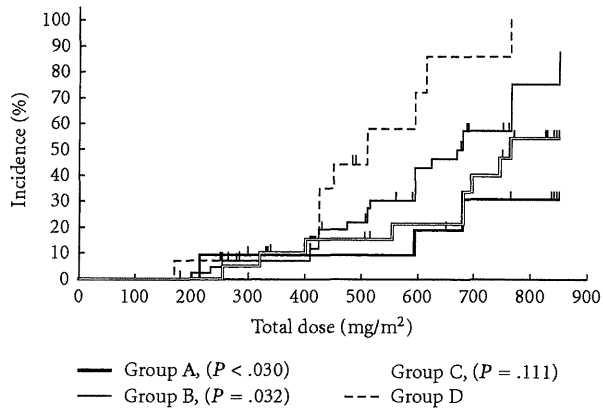

Figure 2: Kaplan-Meier analysis of Grade 2 or worse peripheral neuropathy in relation to the total dose of oxaliplatin. Group A, GJG; Group B, calcium gluconate (Ca) and magnesium sulfate (Mg); Group C, GJG + Ca/Mg; Group D, no therapy. P-values are for comparison to Group D with the log-rank test.

3.4. Treatment Failure and Discontinuation. The TTF was ~2 months longer in the groups given GJG than in the groups without GJG (Figure 4). The rate of discontinuation of treatment due to tumor progression was 18.2% and 20.5% in patients that did not receive Ca/Mg (Groups A and D, resp.), but only 7.1% and 4.8% in those that received Ca/Mg (Groups B and C, resp.) (Table 5). In Group A, no patients discontinued therapy due to peripheral neurotoxicity; in the other groups, the rates ranged from 19.0% to 42.9%. However, Group A showed a higher discontinuation due to hematological toxicity than the other groups. There were no discontinuations due to patient refusal or change of therapy in the groups administered GJG. Also, in Group A, nearly half the patients continued the treatment throughout the study (Table 5).

3.5. Tumor Response. The response rate was 54.5% (6/11) in Group A, 35.7% (5/14) in Group B, 42.9% (9/21) in Group C and 45.5% (20/44) in Group D. The disease control rate (stable disease or better) was 90.9% (10/11) in Group A, 71.4% (10/14) in Group B, 90.5% (19/21) in Group C and 88.6% (39/44) in Group D (Table 6).

Table 5: Reasons for discontinuation of therapy.

	Group A, Pts (%)	Group B, Pts (%)	Group C, Pts (%)	Group D, Pts (%)	Total, Pts (%)
Progressive disease	2 (18.2)	1 (7.1)	1 (4.8)	9 (20.5)	13 (14.4)
Others					
Neuropathy	0	6 (42.9)	4 (19.0)	10 (22.7)	20 (22.2)
Myelosuppression	2 (18.2)	1 (7.1)	0	3 (6.8)	6 (6.7)
Allergy	0	0	2 (9.5)	3 (6.8)	5 (5.6)
Other toxicities	1 (9.1)	2 (14.3)	7 (33.3)	9 (20.5)	19 (21.1)
Resection	1 (9.1)	2 (14.3)	3 (14.3)	1 (2.3)	7 (7.8)
Patient refusal	0	1 (7.1)	0	0	1 (1.1)
Change of therapy	0	0	0	5 (11.4)	5 (5.6)
Continuing	5 (45.5)	1 (7.1)	4 (19.0)	4 (9.1)	14 (15.6)

Group A, GJG; Group B, Ca/Mg; Group C, GJG + Ca/Mg; Group D, no therapy.
GJG, goshajinkigan; Ca, calcium gluconate; Mg, magnesium sulfate.

Table 6: Tumor response to treatment (RECIST).

	Group A	Group B	Group C	Group D
CR	0	0	0	0
PR	6	5	9	20
SD	4	5	10	19
PD	1	3	1	3
NE	0	1	1	2
Response Rate	6 (54.5%)	5 (35.7%)	9 (42.9%)	20 (45.5%)
(CR+PR)	(P = .589)	(P = .522)	(P = .844)	
Disease Control Rate	10 (90.9%)	10 (71.4%)	19 (90.5%)	39 (88.6%)
(CR+PR+PD)	(P = .829)	(P = .121)	(P = .823)	

Group A: GJG, Group B: Ca/Mg, Group C: GJG+Ca/Mg, Group D: no therapy.
CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease; NE: no evaluate.
P-values are for Groups A, B, and C versus. Group D by the χ^2-test.
GJG: goshajinkigan, Ca: calcium gluconate, Mg: magnesium sulfate.

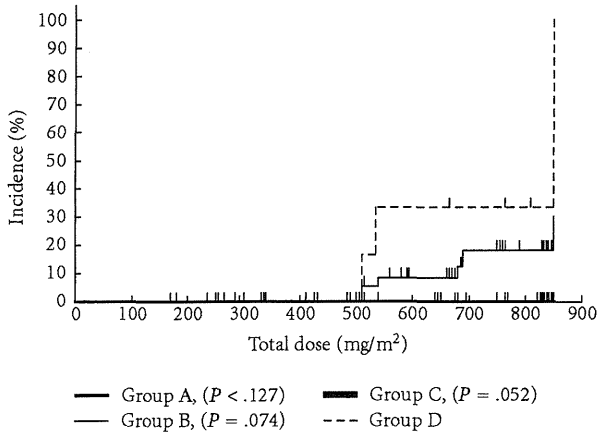

Figure 3: Kaplan-Meier analysis of Grade 3 peripheral neuropathy in relation to the total dose of oxaliplatin. Group A, GJG; Group B, calcium gluconate (Ca) and magnesium sulfate (Mg); Group C, GJG + Ca/Mg; Group D, no therapy. P-values are for comparison to Group D with the log-rank test.

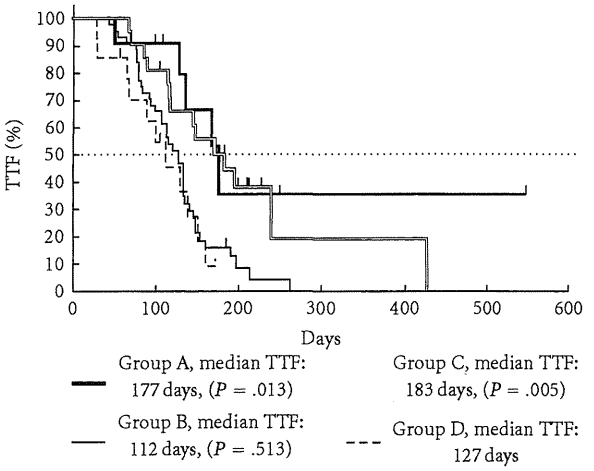

Figure 4: Kaplan-Meier analysis of TTF. Group A, GJG; Group B, calcium gluconate (Ca) and magnesium sulfate (Mg); Group C, GJG + Ca/Mg; Group D, no therapy. P-values are for comparison to Group D with the log-rank test.

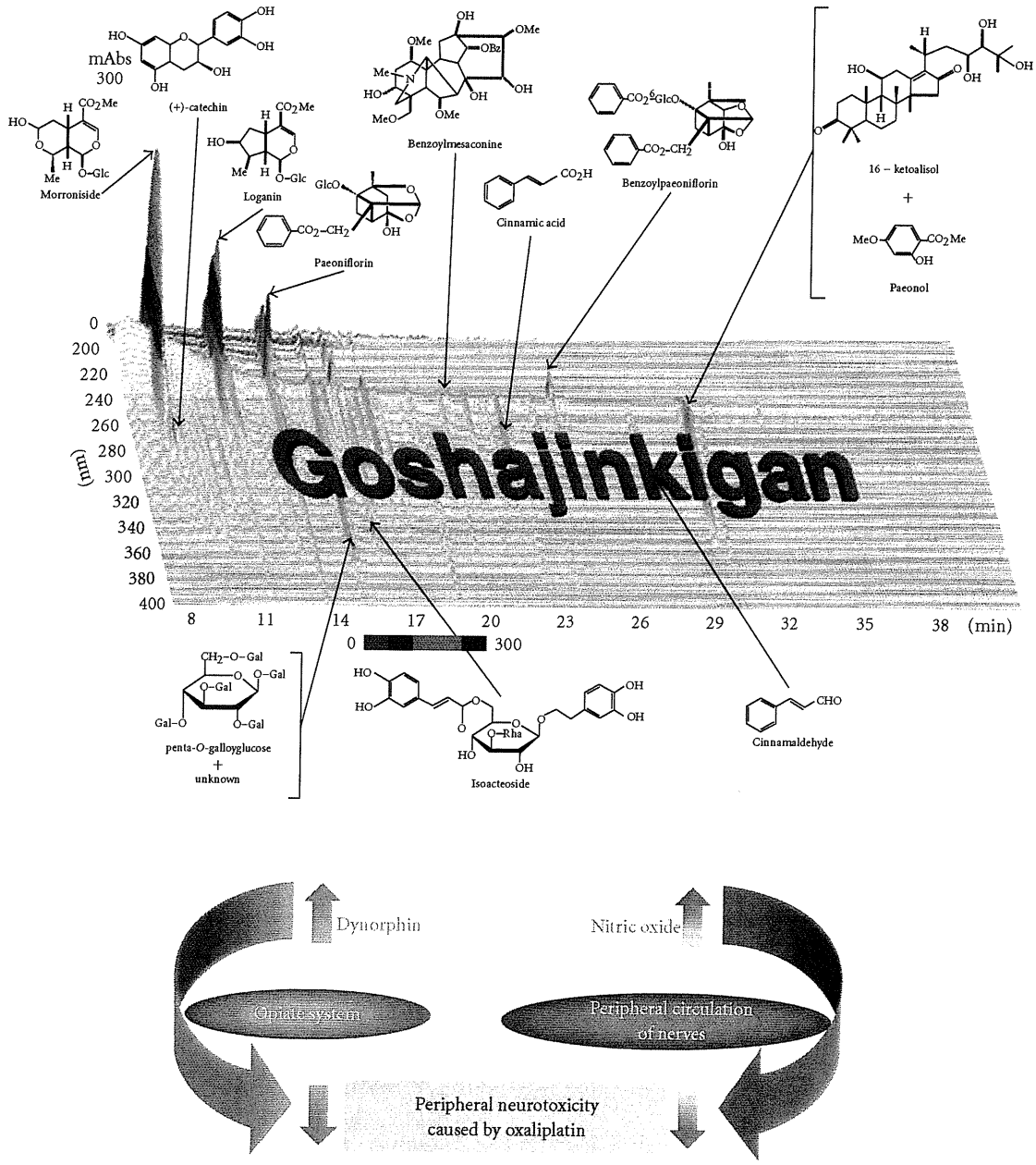

FIGURE 5: Two mechanisms of pharmacological actions of GJG for peripheral neurotoxicity. This shows a representative 3D high performance liquid chromatography of the GJG formulation. Two mechanisms are suggested by which GJG may alleviate peripheral neurotoxicity caused by oxaliplatin.

4. Discussion

Peripheral neurotoxicity is a characteristic adverse effect of oxaliplatin [20]. It is a major obstacle to continuing treatment with regimens that contain this agent; for example, FOLFOX. The main symptoms of peripheral neurotoxicity are typically paresthesia and dysestheia of the extremities induced by exposure to cold. These symptoms occur in 85%–95% of patients, and the symptom duration becomes longer with increasing repetitions of chemotherapy courses. It has been shown that an increase in the total dose of oxaliplatin leads to the occurrence of pain and sensory dysfunction. De Gramont et al. [5] reported that functional impairment occurred in 10% of patients at a total oxaliplatin dose of 850 mg m^{-2}, and this increased to 20% at a total dose of 1020 mg m^{-2}. Thus, it is important to control peripheral neurotoxicity in order to allow continued administration of oxaliplatin. However, control of this side effect is difficult, because the mechanisms underlying the development of neuropathy have not been clarified.

Gamelin et al. [6] suggested that a possible mechanism may be the effect of oxalate, a metabolite of oxaliplatin, on neuronal sodium channels. However, based on this hypothesis, treatment with a sodium channel blocker did not achieve satisfactory results [20]. This suggested that sodium channels may only be involved in acute peripheral neurotoxicity. Neuronal damage has also been attributed to the accumulation of platinum in the dorsal root ganglion based on the results from animal experiments [20]. Thus multiple mechanisms may be involved.

GJG is comprised of 10 herbs and each contains numerous active ingredients (Figure 5). Thus, in Western medical terms, GJG is a complex drug, and its overall pharmacological action is difficult to explain. Until recently, many Japanese people harbored prejudice toward Kampo medicine, doubted their efficacy and showed little interest in their mechanisms [21]. This ignorance of the potential benefits of some herbal medicines is hardly a rare phenomenon, because skeptics of herbal medicine abound wherever herbal medications are used, although herbal medicine has been used throughout the world since time immemorial [22]. Two mechanisms have been suggested by which GJG may alleviate peripheral neurotoxicity (Figure 5) [23–25]. The first is that GJG promotes the release of dynorphin, and thus improves numbness/pallesthesia via the opiate system. The second is that GJG promotes nitric oxide production, and thus improves the circulation and the blood supply to the nerves.

The present study was a retrospective analysis of peripheral neuropathy inhibition in patients that received FOLFOX therapy combined with GJG, Ca/Mg, GJG + Ca/MG or no concomitant drug. Although the number of patients was small, we found that the incidence of peripheral neuropathy was markedly lower in the groups that received GJG when compared with those that did not receive GJG; moreover, there was no Grade 3 peripheral neuropathy in the patients given GJG. In addition, more courses of chemotherapy could be given to patients that received GJG than to those not given GJG; thus, the former also received a higher total dose of oxaliplatin. Furthermore, the TTF was longer in patients that received GJG and there were fewer discontinuations due to peripheral neuropathy than for the other groups. In the group of patients given GJG alone (Group A), almost half the patients had continued oxaliplatin therapy throughout the study, and none discontinued treatment due to peripheral neurotoxicity. Group A showed a higher discontinuation due to hematological toxicity than the other groups. As far as we know, it has never been reported that hematological toxicity was observed in the patients who used GJG in Japan. GJG has been safely used for 25 years in Japan for the improvement of symptoms of numbness, cold sensation and pain of the extremities associated with diabetic neuropathy [12–15]. Therefore, hematological toxicity is not likely to have theadverse effect of GJG. The reason why Group A showed a higher discontinuation due to hematological toxicity is probably because Group A patients received higher dose of total oxaliplatin and 5-FU than other groups. In addition, that group had higher tumor response rates and disease control rates than the other groups, indicating that GJG treatment did not impair anti-tumor activity. Based on these results, we concluded that concomitant administration of GJG contributed to the inhibition of peripheral neurotoxicity and prolonged treatment with oxaliplatin.

Several authors have previously reported suppression of peripheral neurotoxicity by Ca/Mg; however, we could not confirm this effect in this study. In fact, the patients that received GJG + Ca/Mg developed worse neuropathy than those that received GJG alone. Accordingly, we suggest that GJG alone (rather than combined with Ca/Mg) may be more effective for suppression of peripheral neurotoxicity. In addition, the tumor response rate was lower in the group that received GJG + Ca/Mg than in the other groups; this suggested that some interaction may have occurred when GJG and Ca/Mg were combined.

A limitation of the present study was that it was a retrospective review. Also, the number of patients was small, and some of the baseline characteristics differed between the groups. Nevertheless, our findings suggested that the peripheral neurotoxicity of oxaliplatin could be suppressed by administration of GJG. It will be necessary to confirm the usefulness of GJG by performing larger prospective studies in the future [26].

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The authors would like to express their sincere appreciation for the assistance of Dr. Machiko Satomi, Dr. Keisuke Bando, and Mr. Keiichi Shimizu in the analysis of the data.

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8. Endogenous calcitonin gene-related peptide and adrenomedullin are target peptides for Daikenchuto (Da-Jian-Zhong-Tang), an extracted traditional Japanese medicine -New possibility for Crohn's disease management-

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Abstract. Daikenchuto (DKT) is a Japanese herbal medicine (Kampo) and is a mixture of extract powders from dried *Japanese pepper, processed ginger, ginseng radix* and *maltose powder*, and has been commonly used for treatment of bowel disorders, including postoperative ileus, adhesive bowel obstructions and Crohn's disease (CD) in Japan. Experimental evidence reveals the mechanisms that DKT increases gastrointestinal motility by an up-regulation of the calcitonin gene-related peptide (CGRP) as well as acetylcholine release and plasma motilin. CGRP, a member of calcitonin family peptides, is the most powerful vasoactive substance that is expressed in a large population of sensory neurons supplying the gut, and is considered to serve as an important

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protective mechanism for maintaining mucosal integrity. Moreover, CGRP cross reacts another calcitonin family peptide, adrenomedullin (ADM)(ADM/CGRP system). ADM is ubiquitous in gastrointestinal tract, and plays important role for the regulation of microcirculation, angiogenesis, antifibrosis, antibiosis, and down-regulation of proinflammatory cytokines. DKT attenuated dysfunction of microcirculation, mucosal damage and intestinal adhesion, and inhibited mucosal proinflammatory cytokines, including TNFα, in a CD animal model via up-regulation of ADM/CGRP system. DKT may be a unique therapeutic agent for bowel disorders as an ADM/CGRP system-enhancer.

Introduction

Herbal medicine has been used not only in eastern world but also western world since time immemorial. According to the data from 2007 National Health Interview Survey of 24,000 persons conducted by the Center for Disease Control and Prevention's National Center for Health Statistics, approximately 20% of U.S. adults reported use of herbal supplements [1]. Results from the latest American hospital association annual survey revealed that 60% of the hospitals did not provide complementary alternative medicine (CAM) therapies which include herbal medicine. Probable reasons for the low use of CAM in hospitals included lack of convincing, evidence-based information on herbal medicine for physicians. Over the last few years, the Food and Drug Administration (FDA) began shifting its focus on traditional Japanese medicines (called "Kampo") whose exceptionally high quality and standardized ingredients were noteworthy of their attention.

Traditional Japanese medicines are primarily extract granules and their pharmacological actions have been studied and elucidated at the molecular level contrary to herbal medicinal products from many other less well-regulated countries. Until recently, many Japanese people harbored prejudice toward Kampo medicine, doubted their efficacy and showed little interest in their mechanisms. This ignorance of the potential benefits of some herbal medicines is hardly a rare phenomenon, because skeptics of herbal medicine abound wherever herbal medications are used.

Daikenchuto

Daikenchuto (DKT)(Da-Jian-Zhong-Tang), a distinctively unique traditional Japanese medicine, is the most frequently prescribed traditional medicine in Japan, especially in Japanese gastroenterological surgeons. Those Chinese characters translate as, "to reconstruct strongly the diseased gastrointestinal tract to the health" Fig. 1.

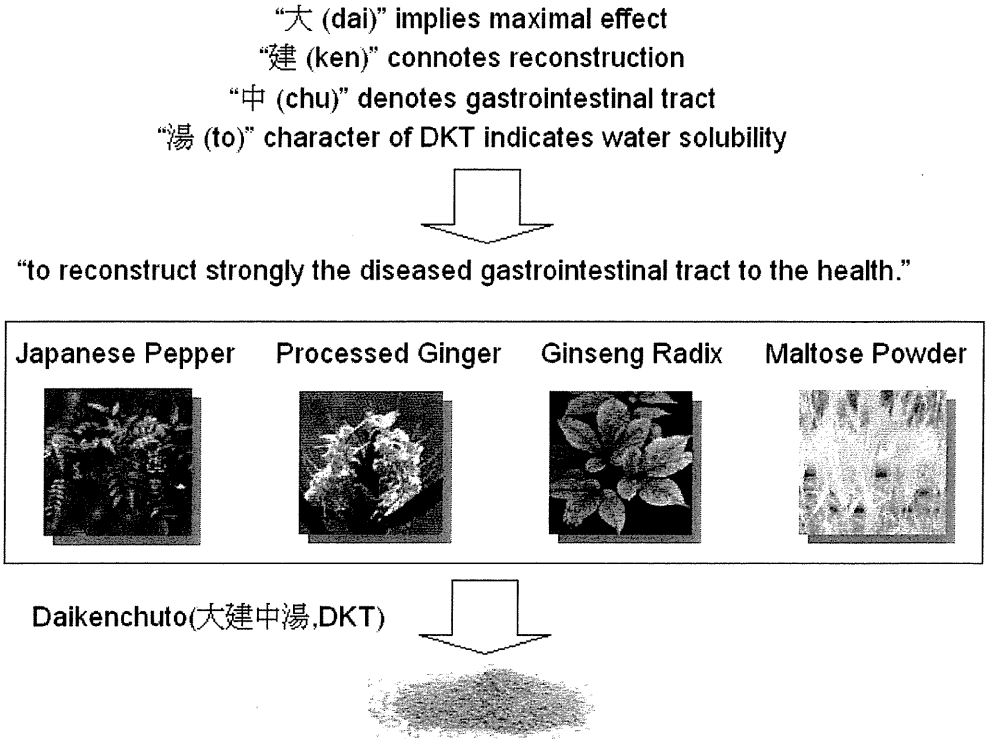

Figure 1. Daikenchuto (Da-Jian-Zhong-Tang).

DKT has been prescribed for abdominal discomfort including pain, distention, and coolness of the abdomen and Japanese government insurance started to cover the medical fee of DKT since 1986. Approximately 500 million DKT sachets (2.5 g) are prescribed annually in Japan, and major adverse events have not been reported to date. DKT has been in the Japanese market for several decades with proven effectiveness for bowel disorders and very few side effects [2]. Although the actual incidences of DKT-related adverse events have not been investigated, 36 cases of minor side effects have been reported by the medical institutions from 1992 to 2007. Our recent study revealed the mechanism of DKT effects at the molecular level [3]. Therefore, a multicenter, double-blind, placebo-controlled study of DKT involving 80% of nationwide university hospitals was launched to explicate the benefits and mechanism of actions of DKT at the clinical level. Results from these large-scale studies are highly anticipated. The objective of this chapter is to introduce the experimental evidence-based information of DKT and to address the concept of appropriate use of DKT.

The formulation of DKT is composed of extract granules of *Japanese pepper*, *processed ginger*, *ginseng radix*, and *maltose powder from rice* (Fig. 1).

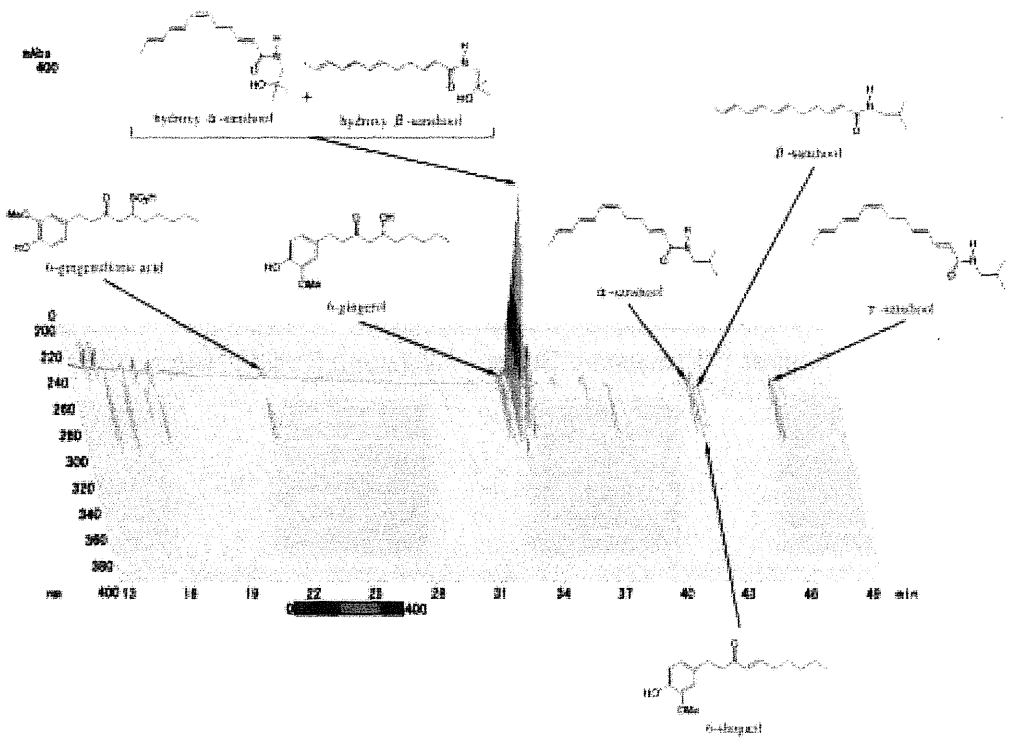

Figure 2. Three-dimensional high-performance liquid chromatography of Daikenchuto.

DKT extract powder (Tsumura Co., Tokyo, Japan) is manufactured as an aqueous extract containing 2.2% *Japanese pepper*, 5.6% *processed ginger*, 3.3% *ginseng*, and 88.9% *maltose syrup powder*. As a chief ingredient compromising nearly 90% of the formulation, maltose confers a sweet taste and improves the palatability of formulation. Other benefits of high maltose (a disaccharide) content include low calorie and controlled sweetness to approximately 1/3 of comparable, sweetened products. The standard dosage of DKT is 15 g/day, and the water-soluble nature of DKT, due to its high maltose content, makes this dosage possible.

As shown in Fig. 2, the ingredients of DKT include hydroxy-α-sanshool (*Japanese pepper*), 6-shogaol (*processed ginger*), ginsenoside Rb1 (*ginseng radix*), and maltose, have been identified by three-dimensional high-performance liquid chromatography. Contamination studies have certified DKT to be free of unexpected pharmaceutical ingredients, toxins, pesticides, microbes, and heavy metals.

In the digestive surgery area, DKT has been employed for speeding the recovery from postoperative ileus after abdominal surgery and its clinical efficacy has been well established [4-7].

DKT and calcitonin gene-related peptide (CGRP)

Several important neural mechanisms have been suggested as mediating the increased effective intestinal motility of DKT [8,9]. One of these mechanisms involves in the release of calcitonin gene-related peptide (CGRP), a neuropeptide produced by the sensory neurons of gut. CGRP is one of the most potent mediators of microvascular vasodilatation in human body and its vasodilating effects following stimulated-release from the extrinsic sensory innervation is considered to serve as an important protective mechanism for maintaining mucosal integrity [10-13]. Because blood flow has to not only meet the relatively high metabolic needs of the gastrointestinal tract, but also provide both valuable buffering and a pathway for removal of toxins that may have entered into tissue [14]. Therefore,

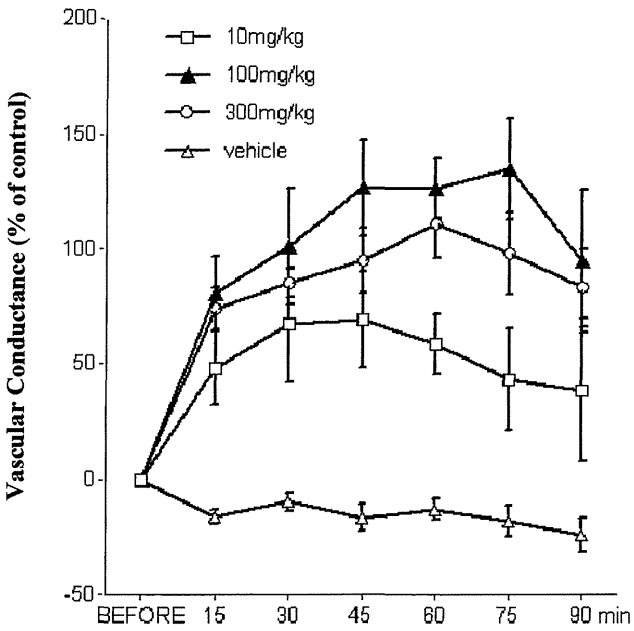

Figure 3. Effects of Daikenchuto (DKT) on colonic vascular conductance (VC) in rats. The rats received an intracolonal injection of vehicle or various doses of DKT (10 and 100 mg/kg). Colonic blood flow was measured by non-contact laser tissue blood flowmetry (LaserMed, ALF21N, Advance Co., Tokyo, Japan) which is a well-characterized technique for the measurement of blood flow in the intestine. Mean colonic vascular conductance (VC) was calculated as the quotient of mean blood flow divided by mean AP and was expressed as ml/mmHg. VC was used as a reliable index of colonic blood flow. VC was calculated every 15 min after the administration of either DKT or vehicle. Data are mean ± S.E.M (n = 8). —△— $P<0.01$ vs. all DKT, —□— $P<0.01$ vs. 100 mg/kg and 300 mg/kg, respectively.

maintaining or increasing blood flow is thought to be a central element in protecting the gastrointestinal tract and even in the prevention of intestinal adhesions resulting from inflammation [15,16].

We have reported that intraduodenal [17] or intracolonic [3] administration of DKT to normal rats increases small or large intestinal blood flow in a dose-dependent manner (Fig. 3). Nervous mechanisms are important for the regulation of gastrointestinal blood flow (Fig. 4). A number of neuropeptides such as CGRP, VIP and SP has been localized immunohistochemically in sensory nerves innervating various viscera, including the gastrointestinal tract [18-23]. Exogenous application of these peptides has been shown to dilate arterioles [13,24,25]. Our study showed that CGRP receptor antagonist, CGRP (8–37), completely abolished DKT-induced hyperemia, whereas the VIP receptor antagonist, [4-Cl-DPhe6, Leu17]-VIP, and SP receptor antagonist did not attenuate the hyperemic response [3](Fig. 5). The pharmacological study suggests that DKT-induced hyperemia of rat intestine is mediated by CGRP, but neither by VIP nor SP release.

Therefore, these observations strongly suggested that direct intraintestinal administration of DKT directed endogenous CGRP release from the sensory nerve terminals of the mucosa, thereby caused an immediate increase in colonic blood flow [3]. Moreover, the results from the study by RT-PCR

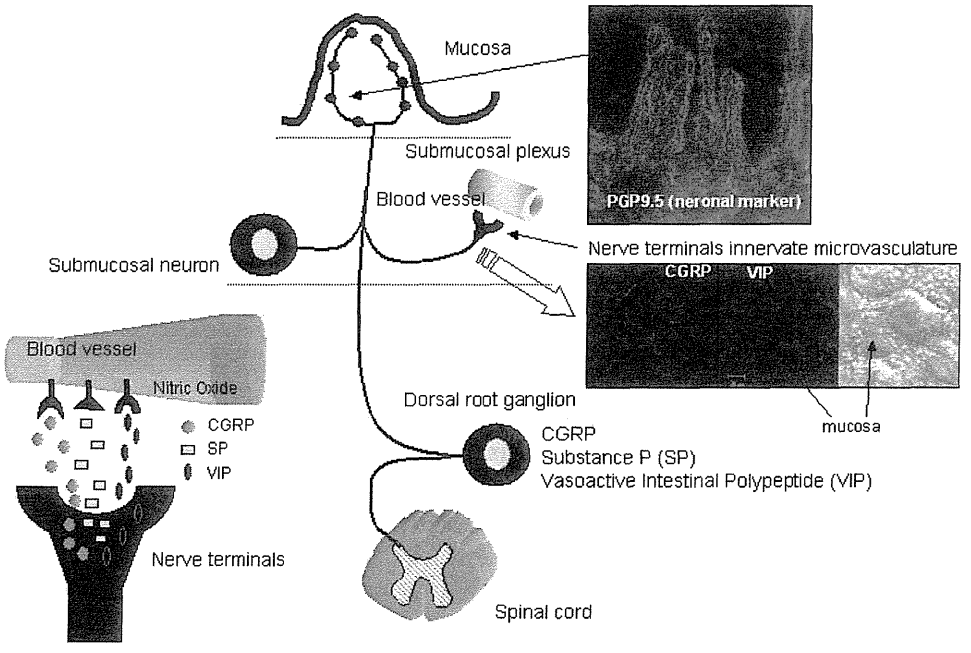

Figure 4. Schematic representation of the neuropeptides-containing sensory neurons in the intestine.

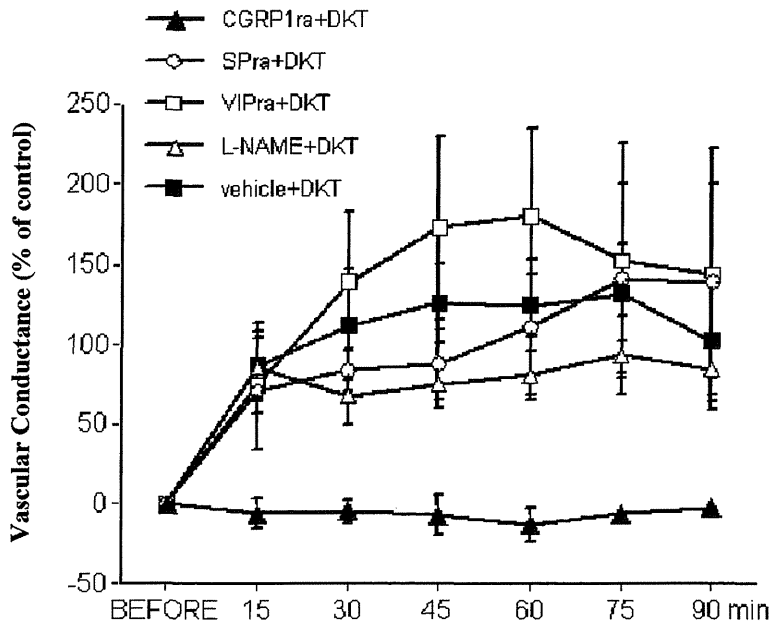

Figure 5. Effects of antagonists on DKT-induced hyperemia. Daikenchuto (DKT, 100 mg/kg) significantly increased colonic vascular conductance (VC) (closed circle). Calcitonin gene-related peptide (CGRP) 1 receptor antagonist completely suppressed the DKT hyperemia (closed triangle). Nitric oxide (NO) blocker, N^G-*nitro*-L-arginine methyl ester (L-NAME), also, but only partially, suppressed VC between at 45 min and at 60 min (open circles). DKT (100 mg/kg) significantly increased colonic VC (closed circles). Vasoactive intestinal polypeptide (VIP) receptor antagonist, *[4-Cl-DPhe6, Leu17]-VIP* and substance P (SP) receptor antagonist, *spantide* did not affect the hyperemic response (open squares and open triangles, respectively). VC is expressed as the quotient of mean blood flow divided by mean AP and was expressed as ml/mmHg. Data are mean ± S.E.M (n = 8). ▲ $P<0.01$ vs. vehicle + DKT, ▲ $P<0.05$ vs. Vehicle + DKT ; CGRP1ra: CGRP1 receptor antagonist *CGRP(8–37)*(45 μg/kg), Spra: SP receptor antagonist *spantide* (100 μg/kg), VIPra: VIP receptor antagonist, *[4-Cl-Dphe6, Leu17]-VIP* (15 μg/kg/h), N^G-*nitro*-L-arginine methyl ester: L-NAME (200 μg/kg).

revealed that DKT had an up-regulatory effect on CGRP [3](Fig. 6). However, the increase in intestinal blood flow by DKT was not observed with intra-gastric administration of DKT, suggesting that the actions of DKT to produce this effect were involved locally rather than systemically (unpublished data).

Another important factor for understanding the mechanism of actions of DKT is through study of the receptors involved. CGRP binds to the receptor termed calcitonin receptor-like receptor (CRLR), a receptor with seven

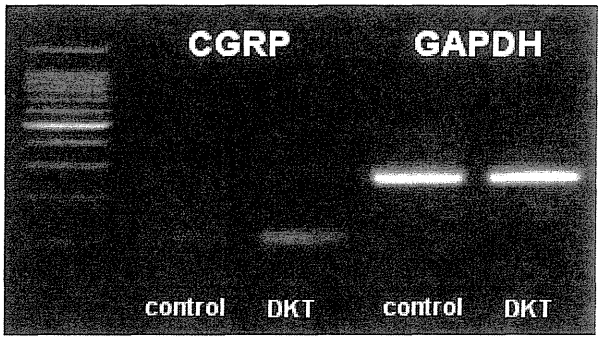

Figure 6. Evaluation of CGRP mRNA from the rat colon after DKT administration. The panel shows representative photos of gel electrophoresis of RT-PCR products and a molecular marker (M), 100 bp DNA ladder. The panel exhibits colonic CGRP mRNA (102 bp) products from the rat colon of vehicle control or Daikenchuto (DKT).

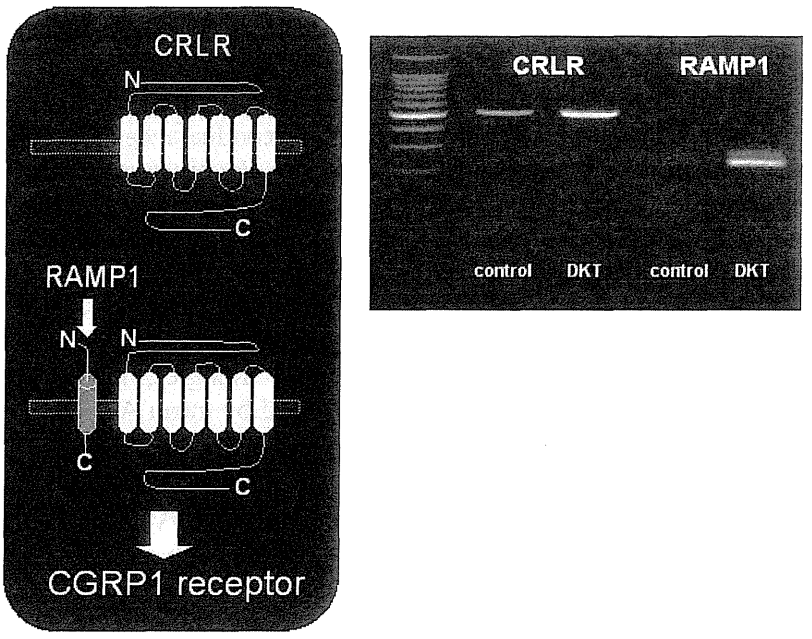

Figure 7. a: Diagrammatic representation of CRLR (show in yellow) and RAMP1(shown in red). Model illustrating the CGRP receptor and showing the interactions between the 7-transmembrane domain G-protein-coupled receptor known as the calcitonin receptor-like receptor (CRLR) and the single-domain receptor-associated membrane protein 1 (RAMP1). **b:** Evaluation of CRLR and RAMP1 mRNA from the rat colon after DKT administration. The right panel shows representative photos of gel electrophoresis of RT-PCR products and a molecular marker (M), 100 bp DNA ladder. The panel exhibits colonic CRLR mRNA (504 bp) and RAMP1 mRNA (230 bp) products from the rat colon of vehicle control or Daikenchuto (DKT).

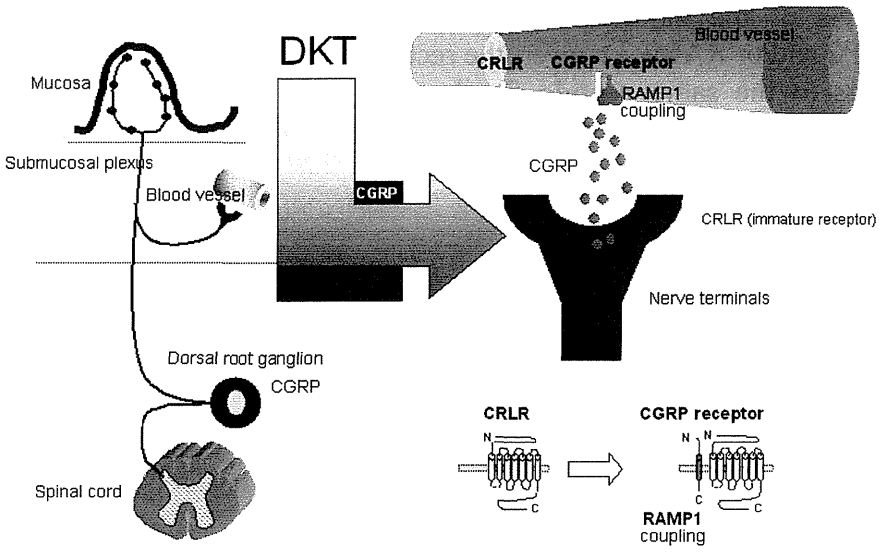

Figure 8. Schematic illustration of the mechanisms of action of DKT locally on sensory neurons of the gut and DKT activates the endogenous calcitonin gene-related peptide (CGRP) system.

transmembrane domains [26, 27]. This receptor can be considered an "immature" receptor with various patterns of expression. This receptor "matures" to the CRLR through the binding of receptor activity modifying protein 1 (RAMP1), a specific type of modulating membrane protein from the family of RAM proteins [28](Fig. 7). Therefore, to confirm the existence of a CGRP1 receptor, it is necessary to determine the existence of not only CRLR but also RAMP1. Our RT-PCR study revealed that DKT had an up-regulatory effect on CRLR and RAMP1 [3](Fig. 7). These lines indicate that when DKT firstly stimulates CGRP release from sensory nerve endings at mucosa, secondarily up-regulates CRLR and RAMP1, and develops up-regulation of CGRP receptor as well as endogenous CGRP up-regulation. Thus, DKT may be used as an endogenous CGRP up-regulator for intestine (Fig. 8).

Ablation of sensory neurons containing CGRP resulted in a marked increase in the severity of inflammation in experimental colitis [29]; inhibition of endogenous CGRP in the colon also increased mucosal damage in experimental models of Crohn's disease [29, 30]. Therefore, we hypothesize that DKT has therapeutic effect on Crohn's disease via up-regulation of endogenous CGRP and its receptor component.

DKT and adrenomedullin (ADM)

It is very interesting that the CRLR family can function as a CGRP receptor or as an adrenomedullin (ADM) receptor depending on which

members of the modifying membrane proteins are expressed, binding of RAMP2 and/or RAMP3 convert the receptor to ADM receptors (Fig. 9). ADM belongs to the same peptide family as CGRP and has potent vasodilating effects in the microvascular system [31]. ADM is ubiquitous in GI tract and plays

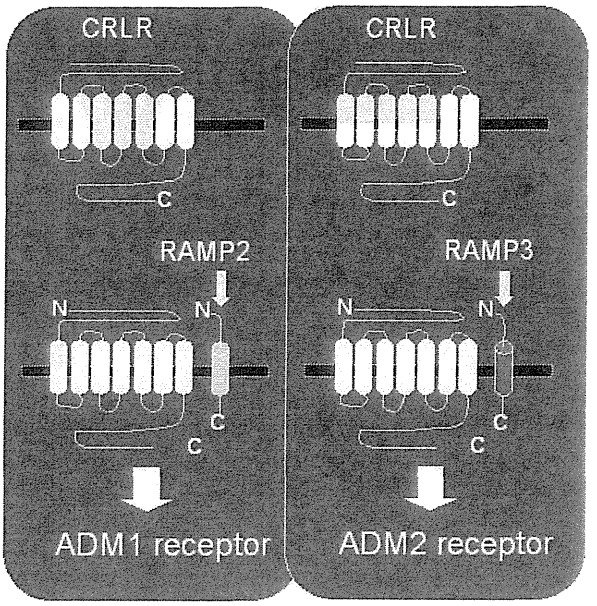

Figure 9. Model illustrating the adrenomedullin (ADM) receptor and showing the interactions between the 7-transmembrane domain G-protein-coupled receptor known as the calcitonin receptor-like receptor (CRLR) and the single-domain receptor-associated membrane proteins (RAMP2 and RAMP3). The subtype of ADM receptor formed by the association of CRLR with RAMP2 is termed ADM1, whereas that formed by association with RAMP3 is termed ADM2.

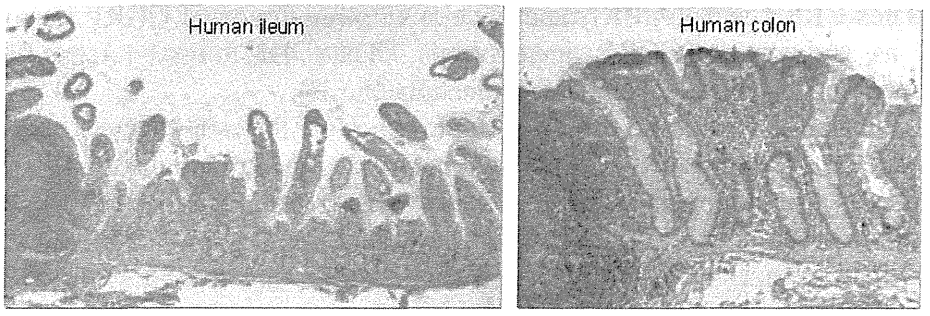

Figure 10. Expression of adrenomedullin (ADM) in mucosal epithelium of intestinal tract. Ileum and colon were obtained from the operated patients, and immuno-histochemical analysis was performed using anti-ADM antibody. ADM was present immuno-histochemically on the apical side of intestinal mucosa surface columnar epithelia.