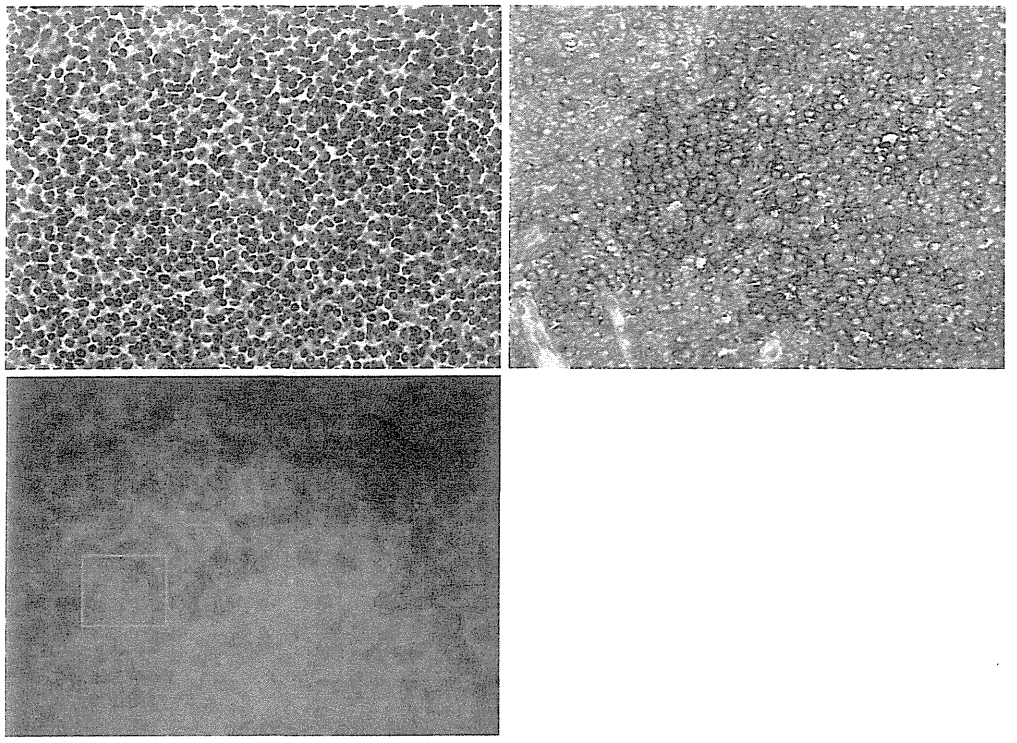

Fig. 4A. The tumor portion was composed of small round cells (H.E. × 400).
B. Immunohistochemical finding shows positive staining for CD99 (× 400).
C. Fish test of the tumor shows separated signal.

A | B
C |

手術所見：腹腔内に少量の血性腹水を認めた．腫瘍は，横行結腸が原発と考えられた皮膜に包まれた多房性腫瘍であり，横行結腸部分切除術を施行し腫瘍を摘出した．

術後病理組織学的検査：腫瘍は一部にmyxomatousな変化や出血を認める，多孔状の腫瘍であった．H.E.染色で特有な構築は認めず均一な小円形細胞が密集していた（**Fig. 2A**）．免疫染色はCD99陽性（**Fig. 2B**），Vimentin陽性で，c-kitは比較的多くの細胞に陽性であった．その他，サイトケラチンAE1/3，Calretinin，S100，CD34，CD10，CD56，Inhibinαはいずれも陰性であった．また，EWSR1 (22q 12) Break probe (POSEIDON) を用いてFISH検査を行ったところ，分離シグナルが検出され(**Fig. 2C**)，ES/pPNETと診断した．

術後経過：高齢であるため術後補助化学療法は行わなかった．術後約1年後の平成23年4月に腹腔内再発に対して再手術を施行した．

症例2：38歳，女性．

主訴：左胸部痛．

現病歴：2010年9月，左胸部痛を主訴に受診．PET検査で左腎腹側にFDGの集積を認める不整な腫瘍を多数認め精査加療目的に当科入院となった．

腹部造影CT検査：左腎臓の腹側に径10cm大の境界明瞭な不整形腫瘍(**Fig. 3A**)と，周囲の腸間膜(**Fig. 3B**)，腹壁にも多数の腫瘍を認めた（**Fig. 3C**)．

以上より腹膜播種を伴うGISTが強く疑われた．主腫瘍が10cmと大きく，確定診断を行う意味も含め手術を施行した．

手術所見：腹腔内に少量の血性腹水を認めた．腫瘍は横行結腸脾彎曲部から発生した径10cmの柔らかい腫瘍であり，腸間膜，大網，腹壁に播種病変を認めた．横行結腸部分切除術にて腫瘍を摘出し，播種病変は可及的に切除した．

術後病理組織学的検査：H.E.染色でNC比の大きい比較的小型で均一な細胞が瀰漫性に増生した（**Fig. 4A**)．免疫染色はCD56，CD99（**Fig. 4B**)，synaptophysin，CD57，NSE陽性で，CD3，CD20，AE1/AE3，EMA，Desmin陰性であった．S-100 protein，LCA，CK7陰性であった．症例1と同様にEWSR1 (22q 12) Break probe (POSEIDON) を用いてFISH検査を行ったところ，分離シグナルが検出され(**Fig. 4C**)，ES/

Table 1. Cases of Japanese ES/pPNET arising from the abdominal cavity

Age/Sex	Symptom	Origin	Size (cm)	Therapy	Prognosis	Recurrence
41/M	Hypogastralgia	Omentum	7.0×5.0	Partial resection Chemotherapy	4M/Death	Local recurrence Dissemination
40/M	Hypogastralgia	Small intestinal mesentery	11×8.0	Partial resection Chemotherapy	7M/Death	Local recurrence Dissemination
24/F	Hypogastralgia	Small intestinal mesentery /Peritoneum	6.0	Surgery Chemoradiation	10M/Death	Dissemination
52/M	Abdominal distention	Mesentery	13×12	Resection	5M/Death	Dissemination
37/M	Abdominal distention	Pelvis/Omentum	Unknown	Partial resection	Unknown	Dissemination
24/F	Abdominal distention	T-colon	12×10	Resection	20M/Survival	None
49/M	Hypogastralgia	Small intestinal mesentery	3.5×2.0	Partial resection	1M/Death	Dissemination
41/M	Hypogastralgia	Small intestinal mesentery	9.0×8.0	Probe laparotomy	1M/Death	S-colon/Sacrum Invasion
59/M	Abdominal mass	D-colon	11	Resection	7M/Death	Local recurrence Dissemination
20/F	Epigastralgia	Small intestinal mesentery	15×13	Bypass surgery Radiation	2M/Death	Dissemination
78/F	Chest pain	T-colon mesentery	13×8.0	Total resection	12M/Survival	Local recurrence Dissemination
38/F	Chest pain	T-colon mesentery	9.4×7.5	Total resection Chemotherapy	12M/Survival	None

pPNET と診断した．

術後補助化学療法：VDC-IE（Vincristine＋Cyclophosphamide＋Doxorubicin＋Ifosfamide＋Etoposide）による術後補助化学療法を 8 コース施行した．術後 1 年の現在，再発を認めず経過良好である．

考　察

ES は，1921年に小児や若年者の骨を原発とした未分化な小円形細胞からなる悪性腫瘍として Ewing により最初に報告された[1]．その後，骨原発性の ES の病理組織によく似た組織像を示す軟部腫瘍を骨外 ES として1975年 Angervall&Enzinger らが報告した[2]．一方，1918年に Stout によって花冠状構造を伴った尺骨原発の小円形細胞腫瘍が報告された後[3]，軟部組織の神経外胚葉への分化を特徴とする腫瘍として末梢性未分化神経外胚葉性腫瘍 Peripheral primitive neuroectodermal tumor（pPNET）の存在が報告されるようになった[4]．しかし，これらの腫瘍は，近年の染色体分析や分子生物学の進歩によって骨原発性 ES，骨外原発性 ES，pPNET，また Askin 腫瘍でも t(11；22)（q24；q12）などの共通の染色体転座を有することが明らかになり[5]，これらは一連の疾患として，新 WHO 分類（2002年）において ES と PNET は種々程度に神経外胚葉への分化を示す円形細胞からなる同一の腫瘍と定義され ES/pPNET として 1 項目に認識されるようになった[6]．また，発生部位も骨や軟部組織だけでなく全身のあらゆる臓器から生じることが明らかになってきている．

軟部組織腫瘍の中で ES/pPNET の発生は 4～7％とまれであり[7][8]，腹腔内原発の ES/pPNET は本邦報告が10例と極めてまれな疾患である[9]．自験例を含めた12例の検討では，男女比は 7：5，平均年齢は41.9歳（20～78歳）であり，従来の ES/pPNET が小児から若年者に多く発生するのに比べて高齢で発生している．ES/pPNET の主な症状は腹痛，腹部膨満感などの腫瘍の増大に関連した症状であるが，小腸穿孔や腸重積による症状も認められている．発生部位，腫瘍最大径，腹膜播種に関しては現在までに報告されているものと同様であった．治療に関しては，外科的切除術が全例に施行されているが，多くは部分切除術であった．術後追加治療としては，術後補助化学療法や放射線療法が施行されていた．予後に関しては，完全切除された 1 例を除いて10カ月以内に死亡しており予後不良であった（**Table 1**）．しかし，われわれの症例は 2 例とも術後経過 1 年以内と短いが，症例 1 では再発を認めているが存命中であり，症例 2 は再発を認めず経過中である．最近国内で，限局性 Ewing Sarcoma Family Tumor（ESFT）に対する VDC-IE 療法の臨床試験や再発，転移を有した ESFT 症例への Irinotecan＋cisplatin 併用療法等の複数の臨床試験が進行中であり，エビデンスとして確立されたものではないが，ES/pPNET に対する治療は，初回手術での完全切除は勿論重要であり，術後補助化学療法や放射線治療などの

集学的治療が必要であると考える。また，完全切除が可能な状態での早期発見が望まれるため腹腔内原発のES/pPNETという疾患はまれではあるが腹腔内腫瘍の鑑別に挙げることも必要であると思われる。

結　語

腹腔内原発の極めてまれなES/pPNETを2例経験したので文献的考察を加え報告した。

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TWO CASE REPORTS OF EWING SARCOMA WITH PERIPHERAL PRIMITIVE NEUROECTODERMAL TUMOR (ES/pPNET) ARISING FROM ABDOMINAL CAVITY

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We report two rare cases of Ewing sarcoma with peripheral primitive neuroectodermal tumor (ES/pPNET) arising from the abdominal cavity in a 78-year-old female patient and a 38-year-old female patient. In Japan, ES/pPNET arising from the abdominal cavity is a very rare disease. We could only find 10 reports in Japan. We experienced two such cases, and put these dates in order and suggest that perfect surgical resection is very important together with postoperative chemotherapy.
Key words : Ewing sarcoma, pPNET, intraabdominal tumor

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ORIGINAL ARTICLE

The Kampo medicine, Goshajinkigan, prevents neuropathy in patients treated by FOLFOX regimen

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Abstract
Background Oxaliplatin is now considered a standard treatment for advanced or unresectable colorectal cancer, but its main dose-limiting toxicity is sensory neuropathy. The OPTIMOX (stop and go) approach offers a reasonable strategy, but the preventive agent is not established. It is reported that the Kampo medicine, Goshajinkigan (GJG), has recently been considered an effective agent for the neuropathy of taxanes and for vibration sensation in patients with diabetic neuropathy. The aim of this study was to clarify the efficacy of GJG for peripheral neuropathy associated with oxaliplatin therapy.
Patients and method From 2007, 45 patients treated with modified FOLFOX6 for non-resectable or recurrent colorectal cancer participated in the study. Twenty-two patients (GJG group) received oral administration of 7.5 g/day of GJG every day during mFOLFOX6 therapy and 23 patients (control group) did not receive GJG. Neuropathy was evaluated during every course according to DEB-NTC (Neurotoxicity Criteria of Debiopharm).
Results The median number of cycles per patient in the GJG group was 13 (range 4–32), and in the control group was 12 (range 4–28). The cumulative dose of oxaliplatin was 1105 mg/m^2 (GJG group) and 1120 mg/m^2 (control group). The incidence of grade 3 peripheral neuropathy in the GJG group was significantly lower than in the control group ($p < 0.01$, log-rank test). The incidence of grade 3 peripheral neuropathy after 10 courses was 0% in the GJG group and 12% in the control group, and after 20 courses was 33% in the GJG group and 75% in the control group. The percentage of grade 2 and 3 peripheral neuropathy in the GJG group was lower than that in the control group. There were no differences in adverse effects between the two groups except for peripheral neuropathy and influence on tumor response.
Conclusion The Kampo medicine, Goshajinkigan, is useful in preventing neuropathy in non-resectable or recurrent colorectal cancer patients treated with a FOLFOX regimen.

Keywords Neuropathy · Kampo medicine · Goshajinkigan · Oxaliplatin · Colorectal cancer

Introduction

Oxaliplatin, a third-generation platinum analog, has demonstrated efficacy as first-line chemotherapy in metastatic colorectal cancer [1] and as adjuvant therapy [2]. Although all platinum analogs are potentially neurotoxic, oxaliplatin is associated with a unique spectrum of neurologic symptoms. Acute neuropathy develops immediately after infusion, characterized by cold-exacerbated paresthesia, muscle spasms, and fasciculations [1, 3]. Although acute symptoms typically resolve within a week, at higher cumulative doses oxaliplatin induces dose-limiting sensory neuropathy leading to sensory ataxia and functional impairment [1, 3]. Severe oxaliplatin-induced neuropathy occurs in 10–20% of

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patients receiving over 750–850 mg/m^2 [1, 2]. Neuropathy limits treatment tolerability, often necessitating treatment delay or cessation, and neuropathic symptoms may persist for a long time [4, 5].

The OPTIMOX (stop and go) approach [6] offers a reasonably good strategy, but attempts to prevent oxaliplatin-induced neuropathy have not been successful. Gamelin et al. [7, 8] 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 glutathione [9], *N*-acetylcysteine [10], xaliproden [11], carbamazepine [12], or glutamine [13], but a preventive agent for oxaliplatin-induced neuropathy has not yet been established. The Kampo medicine, Goshajinkigan (GJG), is composed of 10 natural ingredients and is classified as a drug that affects sensory nerves [14, 15]. Some studies suggested that GJG improved taxanes-induced neuropathy [16] and vibration sensation in patients with diabetic neuropathy [17]. Recently, Kono et al. [18] reported in a retrospective study that GJG was effective for peripheral neurotoxicity of oxaliplatin in patients with advanced or recurrent colorectal cancer.

We conducted the present prospective randomized study to confirm the efficacy of GJG for preventing oxaliplatin-induced peripheral neuropathy in patients with non-resectable or recurrent colorectal cancer who received modified FOLFOX6 (mFOLFOX6) therapy. The aim of this study was to clarify the efficacy of GJG for peripheral neuropathy associated with oxaliplatin therapy.

Materials and methods

Patients

In a study that investigated the neuropathy of various agents, including oxaliplatin, the incidence of more than grade 2 (National Cancer Institute's Common Toxicity Criteria; NCI-CTC) neuropathy was 5% in the Ca/Mg group and 54% in the control group when the mean total dose of oxaliplatin was 500–550 mg/m^2 (equivalent to six cycles at an oxaliplatin dose of 85 mg/m^2) [7]. The number of patients required to reproduce these results was calculated using a type I error (a) of 0.05, a type II error (b) of 0.2, and a control-to-treated data number ratio of 1:1. Therefore, the number of subjects for this study was set at 45 to allow for a 10% dropout rate. From January 2007 to December 2009, a total of 45 advanced or recurrent colorectal cancer patients who received mFOLFOX6 therapy at Tokushima University Hospital were eligible for this study. Patients signed the consent form and fulfilled the following criteria before treatment: Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2, normal bone marrow function (white blood count $\geq$4000/mm^3, platelet count $\geq$100000/mm^3), liver function (serum total bilirubin <1.5 mg/dl), renal function (creatinine <1.5 mg/dl), and heart function (stable cardiac rhythm, no active angina, no clinical evidence of congestive heart failure). Patients were excluded from the study if they had clinical neuropathy, diabetes mellitus, alcoholic disease, or brain involvement, or if they were on vitamin B, magnesium or calcium therapy. Clinical data was collected as follows; age, gender, performance status, primary tumor site, metastatic tumor site, and details of mFOLFOX6 therapy (previous chemotherapy, use of bevacizumab, number of courses, cumulative oxaliplatin dose). Informed consent was obtained from all patients included in the study, which was approved by local ethics committees. This study was registered in UMIN (000002494).

Treatment plan

Therapy was administered on an outpatient basis and patients were premedicated with appropriate antiemetics. Patients were randomly assigned to receive mFOLFOX6 therapy with GJG (GJG group) or without (control group). Random allocation of participants to GJG group or control group was performed by a person not involved in the care or evaluation of the patients. GJG (7.5 g/day divided into 2–3 doses) (Tsumura and Co., Japan), was administered during mFOLFOX6 therapy, given orally before meals or between meals on a daily basis. Other sensory neuromodulatory agents such as calcium–magnesium infusions or antiepileptic-like agents were forbidden. The mFOLFOX6 chemotherapeutic regimen consisted of a 2-h intravenous infusion of oxaliplatin (85 mg/m^2) combined with l-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.

Patient evaluation

Patients enrolled in this study were evaluated at baseline (prior to chemotherapy) and before each course of treatment. The differences between the two groups, GJG group and control group, were evaluated as follows: the incidence of grade 3 peripheral neuropathy, the number of patients in each course, the percentage of grade 2 and 3 peripheral neuropathy in each course, adverse effects (grade 3) except for neuropathy, and influence of tumor response to mFOLFOX6. Peripheral neuropathy evaluations were based on the Neurotoxicity Criteria of Debiopharm (DEB-NTC) [19]. If patients had grade 3 neuropathy, the oxaliplatin dose was reduced to 75% of the previous dose. Adverse effects of

grade 3 except for neuropathy were assessed using the NCI-CTC. Chemotherapy was delayed until recovery if the neutrophil count decreased to less than 1500/L or the platelet count decreased to less than 100000/L. 5FU and oxaliplatin doses were reduced when NCI-CTC grade 3 or 4 non-neurological toxicity occurred. The anti-tumor effect of chemotherapy was assessed by the Guidelines for Evaluation of the Response to Treatment in Solid Tumors (RECIST) [20].

Data analysis

The primary end point of this study was the incidence of grade 3 peripheral neuropathy. The secondary end points were the percentage of grade 2 and 3 peripheral neuropathy in each course, adverse effects except for neuropathy, and tumor response to mFOLFOX6. The assessment of the occurrence of peripheral neuropathy was based on Kaplan–Meier analyses. The two groups were compared with the log-rank test to identify differences in the incidence of peripheral neuropathy. The chi-squared test was used to assess differences in incidence of grade 3 peripheral neuropathy at each course between the two groups. Quantitative data were given as median (range). Comparisons of other clinical data were performed using a chi-squared test, Fisher's exact probability test or Mann–Whitney U test, as appropriate. All statistical tests performed were two-sided and declared at the 5% significance level. All statistical analysis was performed using StatMate version 3 software (Japan).

Results

Patient characteristics

All patients were randomly allocated to the GJG group ($n = 22$) or the control group ($n = 23$). The population in the GJG group consisted of 14 men and 8 women with a median age of 67. The population in the control group consisted of 8 men and 15 women with a median age of 65. The majority of patients in the two groups were PS 0 and 1. The primary tumor sites in the GJG group were 15 colon and 7 rectum, and those in the control group were 16 colon and 7 rectum. The metastatic site was similar in the two groups. There was no statistically significant difference between the two groups based on any of these parameters. The patients' characteristics are listed in Table 1.

Details of mFOLFOX6 therapy

The details of mFOLFOX6 therapy are listed in Table 2. The presence of previous chemotherapy treatment and the use of bevacizumab were similar in the two groups. The median number of chemotherapy courses was 13 (range 4–32) in the GJG group and 12 (range 4–28) in the control group. The median cumulative oxaliplatin (L-OHP) dose was 1105 mg/m^2 (range 340–2720) in the GJG group and 1120 mg/m^2 (range 340–2380) in the control group. There was no statistically significant difference between the two groups based on any of these parameters. In the GJG group, 13 patients discontinued chemotherapy; nine showed progressive disease and four patients experienced an allergic reaction to oxaliplatin. In the control group, 11 patients discontinued chemotherapy; nine showed progressive disease, one had an allergy to oxaliplatin and one patient complained of persistent grade 3 oxaliplatin-induced neuropathy.

Table 1 Patient characteristics

	GJG	Control	p value
n	22	23	
Age	67 (48–77)	65 (52–80)	0.21
Sex			
Male	14 (64%)	8 (35%)	0.1
Female	8 (36%)	15 (65%)	
Performance status			
0	9 (41%)	10 (43%)	0.87
1	10 (45%)	11 (48%)	
2	3 (14%)	2 (9%)	
Primary tumor			
Colon	15 (68%)	16 (70%)	0.82
Rectum	7 (32%)	7 (30%)	
Metastatic site			
Liver	12 (54%)	12 (53%)	0.84
Lung	3 (14%)	4 (17%)	
Local	3 (14%)	1 (4%)	
Lymph node	2 (9%)	3 (13%)	
Other	2 (9%)	3 (13%)	

Table 2 Details of FOLFOX therapy

	GJG ($n = 22$)	Control ($n = 23$)	p value
Previous treatment			
Yes	4 (18%)	4 (17%)	0.75
No	18 (82%)	19 (83%)	
Use of bevacizumab			
Yes	18 (82%)	18 (78%)	0.94
No	4 (18%)	5 (22%)	
No. of courses	13 (4–32)	12 (4–28)	0.87
Cumulative L-OHP dose (mg/m^2)	1105 (340–2720)	1120 (340–2380)	0.87

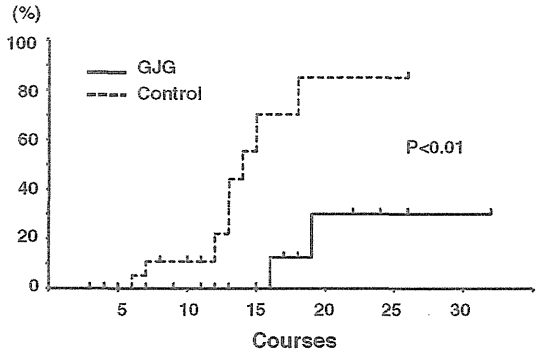

Fig. 1 Kaplan–Meier analyses showed that the incidence of grade 3 peripheral neuropathy occurred significantly less frequently in the GJG group than the control group ($p < 0.01$, log-rank test)

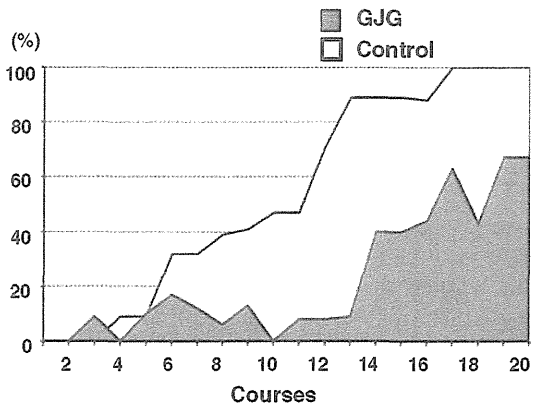

Fig. 2 The percentage of grade 2 and 3 peripheral neuropathy in each cycle was lower in the GJG group than the control group

Effect of GJG on neuropathy

The compliance in the GJG group was 100%. Compliance was checked on the starting day of each course. The number of patients in each course was similar in the two groups. Kaplan–Meier analyses showed that the incidence of grade 3 peripheral neuropathy occurred significantly less frequently in the GJG group than the control group ($p < 0.01$, log-rank test). The incidence of grade 3 peripheral neuropathy after 10 courses was 0% in the GJG group and 12% in the control group, and after 20 courses was 33% in the GJG group and 75% in the control group (Fig. 1). There was no statistically significant difference between the two groups in regard to the incidence of grade 1 or worse and grade 2 or worse peripheral neuropathy (data not shown). The percentage of grade 2 and 3 peripheral neuropathy in each course was lower in the GJG group than the control group (Fig. 2).

Adverse effects and influence on tumor response

Table 3 summarizes adverse effects (grade 3) except for neuropathy. There were no chemotherapy-related deaths during the study. The main toxicities were neutropenia, nausea and diarrhea. In regard to tumor response to mFOLFOX6, no complete response was observed in either group. A partial response was observed in 15 patients (68%) in the GJG group and in 13 patients (57%) in the control group. Stable disease was observed in 5 patients (23%) in the GJG group and in 8 patients (35%) in the control group. The response rate (complete response and partial response) and the disease control rate (complete response, partial response and stable disease) were 68 and 91% in the GJG group and 57 and 92% in the control group, respectively. There were no statistically significant differences in incidence and severity of adverse effects except for peripheral neuropathy and influence on tumor response to mFOLFOX6 between the two groups. The tumor response to mFOLFOX6 is shown in Table 4.

Table 3 Adverse effects (grade 3) except for neuropathy

	GJG ($n = 22$)	Control ($n = 23$)	p value
Neutropenia	3 (14%)	1 (4%)	0.27
Anorexia	0 (0%)	1 (4%)	0.32
Nausea	4 (18%)	2 (9%)	0.34
Vomiting	1 (5%)	1 (4%)	0.97
Diarrhea	2 (9%)	4 (17%)	0.41
Mucositis	2 (9%)	2 (9%)	0.96
All grade 3 toxicity	8 (36%)	8 (35%)	0.84

Table 4 Tumor response to FOLFOX

	GJG ($n = 22$)	Control ($n = 23$)	p value
Tumor response			
Complete response	0 (0%)	0 (0%)	0.86
Partial response	15 (68%)	13 (57%)	
Stable disease	5 (23%)	8 (35%)	
Progressive disease	2 (9%)	2 (8%)	
Response rate	15 (68%)	13 (57%)	0.62
Disease control rate	20 (91%)	21 (92%)	0.96

Discussion

Although the OPTIMOX (stop and go) approach [6] offers a reasonably good strategy, there are several problems, such as the period of use of oxaliplatin and the use of bevacizumab, which are yet to be solved. On the other hand, attempts to prevent oxaliplatin-induced neuropathy have not been sufficiently successful. There are previous randomized controlled studies [9–13, 21] regarding prevention of oxaliplatin-induced neuropathy, including this present report. Five of the seven studies showed the efficacy of the

agent in preventing oxaliplatin-induced peripheral neuropathy. The efficacy of glutamine was reported by Wang et al. [13] and glutathione, a byproduct of glutamine metabolism, was reported by Cascinu et al. [9]. Additionally, Lin et al. [10] reported the efficacy of *N*-acetylcysteine which could increase whole blood concentrations of glutathione in patients with *N*-acetylcysteine supplementation. A major role of glutamine in the prevention of platinum-induced neuropathy has been suggested by several experimental findings. Because glutamine is known to upregulate nerve growth factor (NGF) mRNA in an animal model [22], glutamine supplements may prevent chemotherapy-induced neuropathy via upregulating the NGF level. On the other hand, it has also been hypothesized that high systemic levels of glutamine may downregulate the conversion of glutamine to an excitatory neuropeptide, glutamate, which may also account for the reduced symptoms observed in patients receiving glutamine [23]. Next, a large randomized controlled trial [11] tested xaliproden, a neurotrophic and neuroprotective drug, 11and found that it reduced the risk of grade 3–4 peripheral neuropathy by 39% in metastatic colorectal cancer patients receiving oxaliplatin.

In contrast, two studies of calcium gluconate and magnesium sulfate (Ca/Mg) [21] and carbamazepine [12], the sodium channel blocker, could not show the efficacy of the agent in preventing oxaliplatin-induced peripheral neuropathy. The mechanism of platinum drug neurotoxicity may involve drug accumulation within the peripheral nervous system, especially in the dorsal root ganglia [24]. This suggested that sodium channels may only be involved in acute peripheral neuropathy.

This present study is the first report proving the efficacy of the Kampo medicine, Goshajinkigan, against oxaliplatin-induced peripheral neuropathy using a prospective control study. Neuropathy is the major cause of dose reduction and discontinuation of oxaliplatin treatment [2], with severe neuropathy occurring in 15–20% patients with a cumulative dose of 750–850 mg/m^2 [1, 2]. In the present study, the mean cumulative oxaliplatin dose administered was 1105 mg/m^2 in the GJG group and 1120 mg/m^2 in the control group. Recently, Kono et al. [18] reported in a retrospective study that GJG was effective against peripheral neurotoxicity of oxaliplatin. Additionally, a larger placebo-controlled double-blind randomized phase II study [25] to confirm the usefulness of GJG is taking place in Japan.

A major concern is that GJG might protect tumor cells from the cytotoxic effects of chemotherapy. Although Ca/Mg infusion was suggested to decrease antitumor activity [26], in the current study GJG did not have an influence on tumor response to mFOLFOX6 therapy. Kono et al. [18] reported that the tumor response rate was lower in the group that received GJG + Ca/Mg than in the GJG group and suggested that some interaction might have occurred when GJG and Ca/Mg were combined. Additionally, in the current study GJG did not have an influence on adverse effects except for peripheral neuropathy.

Several mechanisms have been suggested by which GJG may alleviate peripheral neuropathy [27–29]. 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. Recently, Joseph et al. [30] reported that oxaliplatin acted on IB4-positive C-fiber nociceptors to induce an oxidative stress-dependent acute painful peripheral neuropathy. Imamura et al. [31] reported that GJG reduced transmitter proteins and sensory receptors associated with C-fiber activation. This effect may be one of the mechanisms of GJG which prevents oxaliplatin-induced neuropathy.

In regard to combination treatment, Kono et al. [18] reported that the patients who received GJG + Ca/Mg developed worse neuropathy than those who received GJG alone and suggested that GJG alone (rather than combined with Ca/Mg) may be more effective in suppressing peripheral neurotoxicity. Although it will be necessary to confirm the usefulness of combination treatment by performing larger prospective studies in the future, a candidate may be either GJG + glutamine or GJG + xaliproden.

The key weaknesses of this report are as follows: no placebo control, no double-blinding and a small sample. However, Kampo medicines in Japan are strictly monitored by means of three-dimensional high-performance liquid chromatography (3D-HPLC), and therefore their reliability is of a high level. We firmly believe that the result of a placebo-controlled double-blind randomized phase II study [25] to confirm the usefulness of GJG reinforces our findings.

Conclusions

The Kampo medicine, Goshajinkigan, safely reduced the incidence of severe neuropathy by mFOLFOX6 regimen without any adverse influence on the response rate to mFOLFOX6. Therefore, Goshajinkigan is useful in preventing oxaliplatin-induced neuropathy in patients with non-resectable or recurrent colorectal cancer.

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Conflict of interest No author has any conflict of interest.

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Gene Expression Profile Can Predict Pathological Response to Preoperative Chemoradiotherapy in Rectal Cancer

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Abstract. *Background: Preoperative chemoradiotherapy (CRT) has been widely used to improve local control of disease and to preserve the anal sphincter in the treatment of rectal cancer. However, the response to CRT differs among individual tumors. Our purpose of this study was to identify a set of discriminating genes that can be used for characterization and prediction of response to CRT in rectal cancer. Patients and Methods: Seventeen rectal cancer patients who underwent preoperative CRT (40 Gy radiotherapy combined with S-1) were studied. Biopsy specimens were obtained from rectal cancer patients before preoperative CRT and were analyzed by focused DNA microarray (132 genes) and immunohistochemistry. Response to CRT was determined by histopathologic examination of surgically resected specimens and patients were classified as responders (grade 2 or 3) or non-responders (grade 0 or 1). Results: Of the 17 samples, 10 were classified as responders and 7 as non-responders. Seventeen genes were differentially expressed at significant levels ($p<0.05$) between responders and non-responders. All genes showed higher expression in responders as compared with non-responders. The list of discriminating genes included matrix metalloproteinase- (MMP), apoptosis- (nuclear factor kappa light polypeptide gene enhancer in B-cells 2 (NFKB2), transforming growth factor beta 1 (TGFB1)), DNA repair- (topoisomerase 1 (TOP1)), and cell proliferation (integrin, beta 1 (ITGB1))-related genes. In the immunohistochemistry of MMP7, 4 responders were judged as showing overexpression of MMP7. On the other hand, none of the non-responders were judged as showing overexpression of MMP7. Conclusion: Gene expression patterns of diagnostic biopsies can predict pathological response to preoperative CRT with S-1 in rectal cancer.*

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Key Words: Tailored therapy, focused DNA microarray, S-1, chemoradiotherapy, rectal cancer, gene expression profiling.

Locoregional recurrence after resection of rectal cancer is difficult to treat and is associated with severe debilitating symptoms. The prognosis after a local recurrence is poor, with a median survival of 12-18 months (1). Preoperative chemoradiotherapy (CRT) has been widely used as a major treatment modality for locally advanced rectal cancer. Clinical trials have demonstrated that preoperative CRT significantly reduces the risk of local recurrence and toxicity compared with postoperative CRT, but with similar survival rates (2-7). Furthermore, the ability to achieve pathologic downstaging or a complete pathologic response after preoperative CRT is correlated with improved survival, decreased local recurrence, and a higher rate of sphincter-preserving surgery (8-10).

Approximately 40-60% of locally advanced rectal cancer patients treated with preoperative CRT achieve some degree of pathologic downstaging. However, response to CRT differs among individual tumors and there is no effective method of predicting which patients will respond to neoadjuvant CRT. Although responders to CRT have many benefits of CRT, non-responders may unfortunately be subject to the risk of toxicity with no apparent gain. It is therefore of the utmost importance to identify factors prior to preoperative CRT that predict whether a patient is likely to be resistant or sensitive to CRT. The ability to analyze predictive markers of CRT at the levels of RNA, DNA, and protein promises to revolutionize our understanding of the disease process, and it is hoped that the era of genomics, transcriptomics, and proteomics will herald new biomarkers of response to CRT. One strategy, using gene array technology, is to compare the relative gene expression profiles of tumors between responders and non-responders to CRT.

In this report, to predict response to CRT with S-1 before preoperative CRT, we examined the gene expression patterns of diagnostic biopsy samples by customized and focused DNA microarray developed to measure molecular markers involved in response to 5-fluorouracil (5-FU) and other anticancer drugs. The purpose of this study was to define the gene expression patterns for prediction of response to CRT with S-1 and establish tailored therapy for rectal cancer.

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Patients and Methods

Patients and tissue samples. For gene expression profiling, rectal cancer samples were obtained from 20 patients approved to receive preoperative CRT from September 2005 to September 2007 at Tokushima University Hospital. The 20 independent rectal tumor samples included 17 for training and 3 for testing the outcome prediction model, respectively. The patient characteristics and response to CRT are summarized in Table I. We obtained study approval from the Ethics Committee at Tokushima University Hospital and each patient gave written informed consent for samples to be used. Biopsy specimens were prospectively collected during colonoscopic examination from rectal cancer before starting preoperative CRT. Parallel tumor specimens were formalin fixed and paraffin embedded for histologic examination and further specimens were used for RNA extraction. Samples were used for RNA extraction when parallel specimens contained at least 70% tumor cells. Samples were snap-frozen immediately in liquid nitrogen and stored at –80°C until RNA extraction was carried out.

All patients received CRT with a total dose of 4,000 cGy of pelvic irradiation; CRT was administered five times weekly, with a daily fraction of 200 cGy, utilizing a four-field technique. Radiation was delivered concomitantly with S-1, a novel oral fluoropyrimidine inhibitor of dihydropyrimidine dehydrogenase which has a potent radiosensitizing property. S-1 was administered on days of radiation. Surgical treatment was performed 6-8 weeks after the completion of preoperative CRT.

Table I. *Patient characteristics and response to CRT.*

	Training (n=17)	Testing (n=3)
Male/female	13:4	2:1
Age (years), median (range)	60 (47-82)	65 (51-77)
Tumor size (cm), median (range)	4.1 (2.5-7.3)	4.6 (3.5-5.8)
Tumor distance from the anal verge (cm), median (range)	2.0 (0-4.0)	2.5 (1-3.0)
Grade of differentiation		
Well/moderately	17	3
Poorly	0	0
Tumor stage		
T3	15	3
T4	2	0
Nodal stage		
N0	13	2
N1	2	1
N2	2	0
Pathological response		
Grade 0	0	0
Grade 1	7	1
Grade 2	8	2
Grade 3	2	0

Customized DNA microarrays. A customized DNA array (132 genes) has been developed to measure simultaneously molecular markers involved in response to 5-FU and other anticancer drugs. They consist of 30 genes related to pyrimidine/purine/folate metabolism (thymidylate synthase, dihydropyrimidine dehydrogenase, *etc.*), 19 genes related to DNA repair (DNA ligase I, uracil-DNA glycosylase, *etc.*), 8 genes related to drug resistance (P-glycoprotein, topoisomerase I, *etc.*), 7 genes related to apoptosis (P53, *etc.*), 24 genes related to proliferation (vascular endothelial growth factor, histone deacetylase 1, *etc.*), 20 genes related to cell cycle (E2F1, cyclin A1, *etc.*), 21 other genes of DNA methylation, cell adhesion and collagen catabolism (DNA (cytosine-5)-methyltransferase 1, CD34, matrix metalloproteinase 1, *etc.*) and 3 housekeeping genes (glyceraldehyde-3-phosphate dehydrogenase, beta-actin, 40S ribosomal protein S9). Target DNAs made from the 132 genes were immobilized on a glass plate. Each target DNA (200-600 bp) was designed based on sequence homology analysis to minimize cross-hybridization with other genes, and was practically tested by Northern blot. It was possible to relative determine all genes in a single assay. The basic technology of the customized DNA array is almost the same as that of a Stanford-type cDNA microarray.

Frozen tumor tissues were suspended in RLT Buffer (Qiagen, Hilden, Germany) and homogenized using an MM300 Mixer Mill (F. Kurt Retsch GmbH & Co., Haan, Germany). RNA extraction was performed using an RNeasy mini kit (Qiagen). Total RNA quality was judged from the relative intensities of the 28S and 18S ribosomal RNA bands after agarose gel electrophoresis. Purified total RNA (20 μg) was incubated at 70°C for 5 min and cooled on ice. It was reverse-transcribed with a mixture of specific primers and 200 units of PowerScript reverse transcriptase, and incubated at 42°C for 1.5 h. The cDNA was labeled using Cy5 (Cy5 monofunctional reactive dye, Cat. No. PA25001, GE Healthcare–Amersham Biosciences, Piscataway, NJ, USA), and purified by a Nucleo Spin Extract kit (Macherey-Nagel GmbH & Co. KG, Dueren, Germany). Labeled cDNA was hybridized in 6× SSC, 0.2% SDS, 0.01 mg/ml Human Cot-1 DNA and 5× Denhalt's solution for 16 h at 60°C for spotted cDNA arrays. The slides were washed in 2× SSC at room temperature, then 2× SSC with 0.2% SDS at 55-65°C twice, and finally 0.05× SSC at room temperature and scanned using an FLA-8000 Scanner (FujiFilm, Tokyo, Japan). Data was analyzed using an Array Gauge (FujiFilm).

Immunohistochemistry. Immunohistochemical staining was performed on 5-μm thick sections obtained from formalin-fixed and paraffin-embedded tissue blocks of biopsy specimens from rectal cancer patients before starting preoperative CRT. Immunostaining was carried out after heat-based antigen retrieval (20 min, 95°C water bath, citrate buffer [pH 6]) using mouse monoclonal antibody against matrix metalloproteinase-7 (MMP7) (Daiichi Fine Chemical, Toyama, Japan; dilution, 1:50). Automated immunohistochemistry was performed using a Dako Autostainer Plus System (DakoCytomation, Carpinteria, CA, USA) with antimouse IgG EnVision Plus detection kit (DakoCytomation) for secondary and tertiary immunoreactions. Reaction products were developed with diaminobenzidine (DAB), according to standard protocols. Sections were considered to demonstrate MMP7 overexpression if more than 50% of the tumor cells were positively stained. Negative control sections with the omission of the primary antibody were included in each run.

Data analysis. To identify genes that were differentially expressed between the two groups, the data sets were assigned to either responders or non-responders. Response to CRT was evaluated by histopathologic examination and DNA microarray was analyzed. Histopathologic examination of surgically resected specimens was based on a semiquantitative classification system as described in

Table II. *Genes differentially expressed between responders and non-responders.*

No.	Gene symbol	Description	Fold change	*P*-value
1	*MMP7*	Matrix metalloproteinase 7	2.63	0.007
2	*MMP14*	Matrix metalloproteinase 14	2.29	0.013
3	*MMP9*	Matrix metalloproteinase 9	1.86	0.013
4	*MMP1*	Matrix metalloproteinase 1	1.85	0.045
5	*ITGA2*	Integrin, alpha 2	1.78	0.045
6	*NFKB2*	NFK light polypeptide gene enhancer in B-cells 2	1.63	0.028
7	*CTSB*	Cathepsin B	1.48	0.005
8	*ITGB1*	Integrin, beta 1	1.48	0.045
9	*MMP16*	Matrix metalloproteinase 16	1.45	0.045
10	*PLAUR*	Plasminogen activator, urokinase receptor	1.40	0.028
11	*DNMT1*	Ribonucleotide reductase M1 polypeptide	1.38	0.028
12	*DNMT1*	DNA (cytosine-5-)-methyltransferase 1	1.38	0.028
13	*UP*	Uridine phosphorylase 1	1.38	0.036
14	*TOP1*	Topoisomerase (DNA) I	1.34	0.022
15	*TGFB1*	Transforming growth factor, beta 1	1.32	0.045
16	*NDKA*	Non-metastatic cells 1, protein	1.30	0.045
17	*NDKB*	Non-metastatic cells 2, protein	1.09	0.045

NFK: Nuclear factor kappa

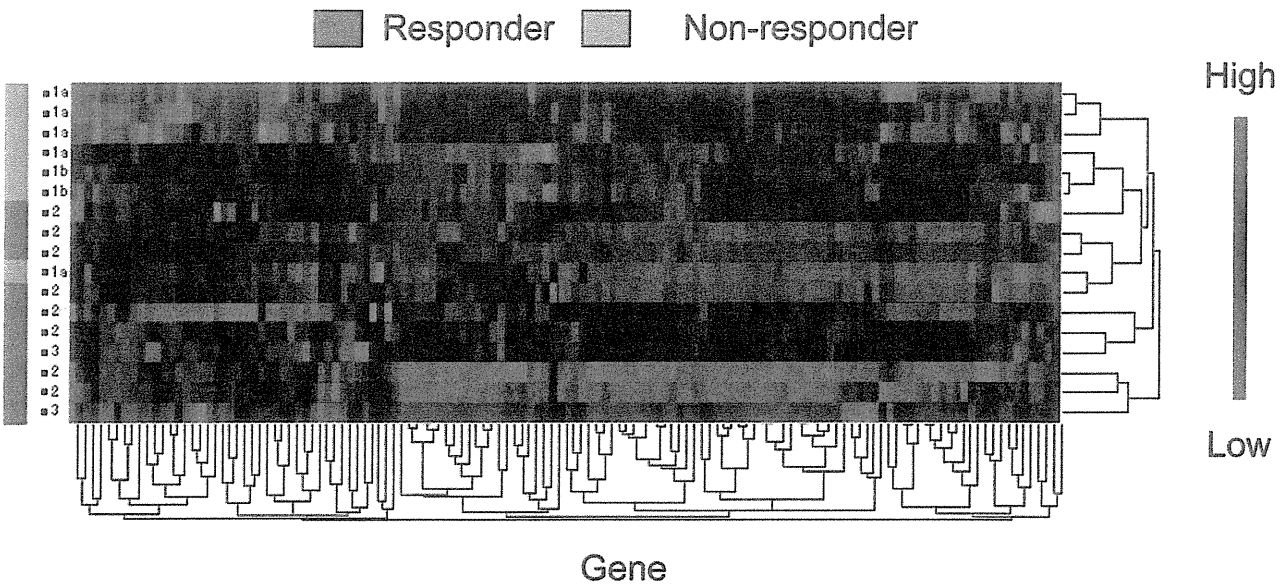

Figure 1. *A hierarchical cluster analysis of 132 genes. Red, Overexpression; green, underexpression. Responders and non-responders were clustered into two distinct groups except for four responder cases.*

detail previously (11). Tumors were classified as "responder" when assigned to the regression grade 2 or 3, and "non-responder" when grade 0 or 1.

Samples from 20 patients were divided into a training set (17 samples) and a testing set (3 samples). Only training samples were used in the DNA microarray analysis to evaluate gene expression. The expression patterns were compared and fold-change value calculated to identify gene markers that can best discriminate between responders and non-responders. Two-dimensional hierarchical clustering was then applied to the log-transformed data with average-linkage clustering with standard correlation as the similarity metric for the discriminating genes that were identified as being differentially expressed between responders and non-responders. Next, using immunohistochemistry in a training set, we evaluated a candidate gene, *MMP7*, detected by DNA microarray analysis as being the most highly overexpressed. Additionally, in a testing set, the gene expression patterns of diagnostic biopsy samples were evaluated by focused DNA microarray before preoperative CRT regarding to the histopathologic examination of surgical specimens.

Table III. *Validation of the gene expression patterns by microarrays.*

Test sample (n=3)	Response to CRT	Overexpressed genes (n)
1	Grade 2	5/17
2	Grade 2	4/17
3	Grade 1	0/17

Statistics. Quantitative data were given as median (range). All statistical analysis was performed using statistical software (JMP 8.0.1., SAS, Cary, NC, USA). A comparison of immunohistochemistry data was performed using Fisher's exact test, as appropriate. The expression patterns in the DNA microarray were compared using unpaired *t*-tests (with Welch's correction for unequal variances). All statistical tests performed were two-sided and declared at the 5% significance level.

Results

Gene expression patterns by microarray in responders and non-responders. Gene expression profiling was established using customized and focused DNA microarray in training samples. There was no significant difference between the training set and the testing set in clinicopathologic factors such as gender, age, histopathologic classification, preoperative tumor stage, response to CRT, and so on. The patient characteristics and response to CRT are summarized in Table I. Among the 17 training samples, 10 were classified as responders and 7 as non-responders, according to the histopathologic examination of surgical specimens. Regarding histopathologic examination of surgically resected specimens, 17 genes were identified that were significantly ($p<0.05$) differentially expressed between responders and non-responders (Table II). All genes showed higher expression in responders as compared with non-responders. The 17 genes were matrix metalloproteinase 7 (*MMP7*), *MMP14, MMP9, MMP1,* integrin, alpha 2 (*ITGA2*), nuclear factor kappa light polypeptide gene enhancer in B-cells 2 (*NFKB2*), cathepsin B (*CTSB*), integrin, beta 1 (*ITGB1*), *MMP16,* plasminogen activator, urokinase receptor (*PLAUR*), ribonucleotide reductase M1 (*RRM1*), DNA (cytosine-5)-methyltransferase 1(*DNMT1*), uridine phosphorylase (UP), topoisomerase 1 (*TOP1*), transforming growth factor, beta 1 (*TGFB1*), nucleoside diphosphate kinase A (*NDKA*), and nucleoside diphosphate kinase B (*NDKB*). Results of a hierarchical cluster analysis of the 132 genes are presented in Figure 1. Responders and non-responders were clustered into two distinct groups except for four responder cases.

Immunohistochemistry of MMP7. MMP7, as a candidate gene, showed the highest fold-change in responders as compared with non-responders in histopathologic examination, and was chosen for validation of DNA microarray data by immunohistochemistry. MMP7 was evaluated by using immunohistochemistry examination in a training set. Four cases out of the responders (n=10) were judged as showing overexpression of MMP7. On the other hand, none of the non-responders (n=7) were judged as showing overexpression of MMP7. There was a tendency for there being a difference in expression of MMP7 between responders and non-responders.

Validation of the gene expression patterns by microarrays. Gene expression profiling (17 genes) was validated using customized and focused DNA microarray in testing samples. Among the three testing samples, two were classified as responders and one as non-responders according to the histopathologic examination of surgical specimens (Table I). One case of the responders showed overexpression of 5 out of 17 genes. Another case in the responders showed overexpression of 4 out of 17 genes. These overexpressed genes included *MMP7* and *TGFB1*. On the other hand, the non-responder case showed no overexpression of any of the genes studied (Table III).

Discussion

Although gene expression patterns have been applied to the outcome prediction of multiple types of cancer, there are few studies to date that have reported the application of DNA array to predict response to CRT using preoperative biopsy tissue samples for rectal cancer. We defined the gene expression patterns for prediction of response to CRT by customized and focused DNA microarray and validated a candidate gene (*MMP7*) by immunohistochemistry.

Regarding DNA microarray, two studies have incorporated microarray analyses to assess gene expression profiles to predict CRT outcome in rectal cancer. In a study by Ghadimi *et al.* (12), 23 pretreatment tumor biopsies were evaluated by cDNA microarrays. The analysis revealed 54 to be genes differentially expressed between responders and non-responders, on the basis of downstaging ($p<0.001$). Using the leave-one-out cross-validation (LOOCV) method, 19 out of 23 patients had their response accurately predicted by their gene expression profiles ($p=0.02$). Using this method, 7 out of 9 responders and 12 out of 14 non-responders were correctly identified. In a validation set comprising 7 different tumor samples, 39 out of the original 54 genes identified from the training set were found to be differentially expressed. In the validation set, the gene expression profile was able to accurately predict response in six out of seven tumors.

Additionally, Rimkus *et al.* (13) evaluated pretreatment biopsies of 43 rectal cancer patients treated with neoadjuvant CRT. The microarray analysis revealed 42 genes to be differentially expressed among responders and non-responders, according to tumor regression grading. These 42

genes were identified from the 50 probe sets with the lowest *p*-values according to the Welch test. Using the LOOCV method, 10 out of 14 responders were correctly predicted, whereas 25 out of 29 non-responders were correctly predicted. In addition, 38 out of the 43 patients were selected randomly for a training set to develop a response classifier. This response classifier was used to predict response status in a small validation set consisting of five patients. The classifier predicted response in the validation-set patients with similar accuracy to the LOOCV method.

Both previous studies (12, 13) reported the ability to accurately determine responders and non-responders on the basis of microarray-determined gene expression profiles. However, between the 54 genes differentially expressed in the Ghadimi study and the 43 genes differentially expressed in the Rimkus study, there was no concordance, not even for a single gene. Furthermore, including our study, there was no concordance for any gene among the three studies. These studies, with a small number of patients, may not have sufficient power to validate the use of microarray-determined gene expression profiles to predict response to neoadjuvant CRT in rectal cancer. Kuremsky *et al.* (14), in a critical review of DNA microarray analysis, reported that although gene array expression data generate interesting results that may lead to the further exploration of candidate genes, the complexity and magnitude makes the results difficult to interpret.

Regarding the prediction of response to CRT using immunohistochemistry, Kuremsky *et al.* (14) reported that the six most commonly researched biomarkers evaluated were p53, epidermal growth factor receptor (EGFR), thymidylate synthase (TYMS), Ki-67, p21, and BCL-2/BAX. There is currently not enough evidence to suggest the clinical application of any biomarker to predict outcome in rectal cancer. We evaluated immunohistochemistry of *MMP7*, as a candidate gene, which showed the highest fold change in responders as compared with non-responders in histopathologic examination. Although MMP7 expression has not been previously described in rectal cancer as a biomarker to predict response of CRT, MMP7 appears to be a candidate biomarker, requiring future investigation.

The expression of *MMP7* in several types of cancer has been confirmed (15-17). The direct interaction of individual MMPs, particularly *MMP7*, with the genes and proteins involved in colorectal cancer development has been shown (18, 19). Specifically, the *MMP7* protein and its mRNA are also consistently expressed in liver metastases of colon cancer (18, 20). Our data suggest that preoperative CRT may be able to improve the prognosis of advanced rectal cancer patients with overexpression of *MMP7*.

Our validation of the gene expression patterns by microarrays revealed *TGFB1*, a gene related to apoptosis. *TGFB1* is a tumor suppressor gene. Barcellos-Hoff *et al.* showed in mice that activation of Tgf-b is an early and sensitive response to irradiation (21). In addition to acting as a tumor suppressor, TGFB has also been shown to have a pro-tumorigenic effect (22). Induction of Ras was shown to decrease the growth-inhibitory response to TGFB (23). There are reports showing that K-ras signaling may play a role in the conversion of TGFB from a tumor suppressor to a tumor promoter (24).

The present study defined the gene expression patterns for prediction of response to CRT with S-1 by customized and focused DNA microarray and validated its ability by immunohistochemistry and microarray using preoperative biopsy tissue samples in rectal cancer. Although the key weaknesses of this study are small sample, retrospective native and unsatisfactory analysis of validation, we evaluated gene expression patterns for prediction of response to CRT with S-1 by customized and focused DNA microarray. A multicenter randomized study for prediction of response to CRT (S-1 *vs.* UFT) by customized and focused DNA microarray is currently in progress. It will be necessary to confirm the usefulness of gene expression patterns for the prediction of response to CRT by larger prospective studies.

Conclusion

Gene expression patterns of diagnostic biopsies can predict pathological response to CRT with S-1 in rectal cancer.

Author Disclosures

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ORIGINAL

Intraperitoneal infusion of paclitaxel with S-1 for peritoneal metastasis of advanced gastric cancer : phase I study

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Abstract : Background : Intraperitoneal administration of taxanes revealed excellent anti-tumor effect for peritoneal metastasis of gastric cancer in some experimental models. The aim of this study is to determine maximum tolerated dose (MTD), dose limiting toxicity (DLT) and recommended dose (RD) of intraperitoneally infused paclitaxel (PTX) with S-1 as a phase I study. Patients and Methods : Eighteen patients with advanced gastric cancer in addition to confirmed peritoneal metastasis using laparoscopy were enrolled in this study. The regimen consists of oral administration of S-1 (Dose 80 mg : BSA< 1.25 m^2, 100 mg : 1.25<BSA<1.5 m^2, 120 mg : BSA>1.5 m^2) for 14 days and intraperitoneal infusion of PTX (Dose escalation : level I : 40, II : 60, III : 80, level IV : 90, V : 100 mg/m^2) at day1 and 14. PTX concentrations in serum and ascites were determined at 4, 8, 12, 24, 48 hours after the infusion, which was repeated twice every 4 weeks. Results : The number of patients were as follows : Level I : 3, Level II : 6, Level III : 3, Level IV : 3, Level V : 3. Grade 3 leukocytopenia was confirmed in 1 (Level II) and 2 (Level V). MTD is 90 mg/m^2, RD is 80 mg/m^2 and DLT is Grade 3 leukocytopenia. The average serum PTX concentrations remained in optimal range except for all 3 of level V patients. In all cohorts, the PTX concentrations in the ascites were approximately 1000 folds higher than those in serum for 48 hours after the infusion. Conclusions : MTD and RD were PTX 90 mg/m^2, 80 mg/m^2, respectively. These findings were supported by pharmocokinetics of PTX J. Med. Invest. 58 : 134-139, February, 2011

Mini-Abstract : *In intraperitoneal infusion of PTX with S-1, DLT was leukocytopenia, MTD and RD were PTX 90 mg/m^2, 80 mg/m^2, respectively. These findings were supported by pharmocokinetics of PTX*

Keywords : *paclitaxel, S-1, intraperitoneal infusion, peritoneal metastasis, gastric cancer*

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INTRODUCTION

Median survival time, even with the best supportive care, for patients with unresectable or metastatic gastric cancer is only 3.1 months (1). Although peritoneum is the most common metastatic site of

advanced gastric cancer, a standard regimen has not been established despite the number of trials and the survival rate is very low.

Recently new chemotherapy agents have been developed. In particular S-1 revealed a high response rate of 49% for advanced gastric cancer in late phase II study (2), which has been widely accepted as a key drug even for adjuvant setting in Japan (3).

Taxanes stabilize and excessively form microtubules, which is a different mechanism from other agents. In phase II study, response rate of paclitaxel (PTX) for advanced gastric cancer was 21% and not affected by differentiation of adenocarcinoma (4, 5). High concentrations approximately 1000 times of PTX in the peritoneal cavity maintained compared with those in serum after intraperitoneal administration because of fat solubility and heavy molecular weight ; 853.92 (6). Excellent pharmacokinetics and anti-tumor effect to the peritoneal dissemination of gastric cancer was reported in the experimental model (7).

It is considered that S-1 and PTX is one of the best combinations for the treatment peritoneal metastasis of gastric cancer. The aim of this study is to determine the appropriate doses and feasibility of intraperitoneal infusion of paclitaxel (PTX) with orally administered S-1.

PATIENTS AND METHODS

Patient eligibility

Patients with peritoneal metastasis of advanced gastric cancer were eligible for this clinical trial. Before initiation of the study, relevant study documentation was submitted to and approved by the responsible ethics committee : the University of Tokushima hospital clinical research Ethical Review Board, Tokushima, Japan.

The guidelines of the World Medical Association Declaration of Helsinki in its revised edition (Edinburgh, Scotland, October 2000) and other applicable regulatory requirements were strictly followed. Written informed consent was obtained from each patient before any study-specific screening procedures were undertaken.

Inclusion criteria

Patients aged 20-75 years, had to have histologically or cytologically confirmed peritoneal metastasis of gastric cancer using laparoscopy under general anesthesia, who had not received abdominal surgery and any prior chemotherapy regimens.

Exclusion criteria

Patients with ischemic heart disease that needed medication, liver cirrhosis, lung fibrosis, pneumonia, intestinal bleeding or other severe complications were excluded.

Treatment plan

An initial laparoscopy was performed under general anesthesia for the patients with advanced gastric cancer histologically diagnosed. Peritoneal metastasis was histologically confirmed by removal of disseminated nodules or peritoneal cytology.

The catheter for intraperitoneal infusion of PTX was passed through the wound of trocar port in the right side of the umbilicus, which was connected to the port implanted in the abdominal wall for the patient diagnosed peritoneal metastasis.

S-1 was orally administered with a fixed quantity (Dose 80 mg : Body Surface Area (BSA) < 1.25 m^2, 100 mg : 1.25 < BSA < 1.5 m^2, 120 mg : BSA > 1.5 m^2) for 14 days. PTX was infused intraperitoneally through the implanted catheter at day1 and 14. Dose of PTX was escalated ; level I : 40 mg/m^2, level II : 60 mg/m^2, level III : 80 mg/m^2 level IV : 90 mg/m^2, level V : 100 mg/m^2. Intraperitoneal PTX with S-1 was repeated two cycles every four weeks.

Adverse events were coded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 3.0. Dose Limiting Toxicity (DLT) was defined two patients had nonhematologic or hematologic grade 3 or greater adverse events. If one patient had Grade 3 or more adverse events, the cohort was expanded to three patients owing to occurrence of a DLT. As a result, the dose of PTX was increased to the level that two patients had a DLT in turn. The Maximum Tolerated Dose (MTD) was defined as one escalation level lower than that DLT was confirmed. Recommended dose (RD) was defined as one level lower than MTD.

Analytic methods and pharmocokinetics

Blood samples for pharmacokinetic analysis were drawn before infusion, at 4, 8, 12, 24 and 48 h after the infusion of PTX. Ascites samples were aspirated through the catheter for PTX infusion at the same time points. High performance liquid chromatography (Ultra-Violet absorbance detector : Ultra-violet of 227 nm in wave length) was used to analyze PTX concentrations of serum and ascites in SRL, Inc

(Tokyo, Japan).

RESULTS

Patient demographics

Patient demographics are shown in Table 1. The 18 patients were enrolled in this study after histologically confirming peritoneal metastasis. Two of the 18 patients had adenocarcninoma cells in peritoneal cytology without macroscopically detected metastatic nodules. Curative operation was not impossible for all 18 patients.

Table 1 : Patient demographics

Sex (male/female)	14/4
Age (years) (median/min/max)	56/49/75
WHO Performance status (0/1)	14/4
Macroscopic types III / IV	10/8
Histological typing well/poorly differentiated	3/15
Positive adenocarcinoma cells in peritoneal cytology	18
Macroscopically detected metastatic nodules	16
Gastrectomy	12

Clinical safety and tolerability

All 18 enrolled patients were evaluated for safety. A summary of the patient- and investigator-reported drug related clinical adverse events is shown in Table 2. Current regimen was generally well tolerated, with 6 patients clinically significant drug-related adverse events. The most frequently reported adverse event was Grade 3 leukocytopenia. Grade 1 or 2 anemia, vomiting and abdominal pain were confirmed.

The 40, 80, 90 and 100 mg/m^2 cohort enrolled three patients. After the one patient had Grade 3 leukocytopenia in 60 mg/m^2 cohort, this cohort was expanded to 6 patients without Grade 3 or more adverse events. Grade 3 leukocytopenia was confirmed consecutively 2 patients in 100 mg/m^2 cohort.

DLT was leukocytopenia, MTD was 90 mg/m^2 and RD was 80 mg/m^2, respectively.

Table 2 : Drug-related adverse events

	Grade 1	Grade 2	Grade 3	Grade 4
BLOOD/BONE MARROW				
Hemoglobin	1 (5.6%)			
Leukocytes			3 (16.7%)	
GASTROINTESTINAL				
Vomiting	1(5.6%)			
PAIN				
Abdominal pain		1 (5.6%)		

Pharmocokinetics of PTX

The average serum PTX concentrations in 40, 60, 80 and 90 mg/m^2 cohort were maintained between the lower limit of cytotoxic effects and upper limit of blood system disorder, which were over upper limit of blood system disorder in all 3 patients of 100 mg/m^2 cohort. In all cohorts, PTX concentrations in the ascites were approximately 1000 folds higher than those in serum for 48 hours after the infusion (Figure 1).

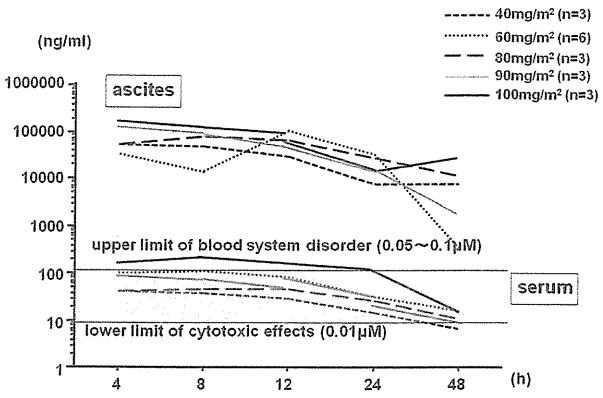

Figure 1 : Pharmacokinetics of PTX
The PTX concentrations in 40, 60, 80 and 90 mg/m^2 cohort remained in the optimal range. In 100 mg/m^2 cohort, the PTX concentrations were over upper limit of blood system. PTX concentrations in the ascites were approximately 1000 times higher than those in serum.

Clinical activity

All 18 patients were evaluated for efficacy. Objective clinical response was assessed according to Response Evaluation Criteria in Solid Tumors (RECIST). The 2 patients had partial response. The 15 patients was recorded as stable disease, however, positive adenocarcinoma cells in peritoneal cytology became negative in 2 patients, remarkable decrease of ascites was found in 2 patients. Down staging according to the 13th Japanese Classification of Gastric Carcinoma was possible in 2 patients (2 : positive cytology became negative). There was one patient classified as having progressive disease.

Gastrectomy was performed for 12 of 18 patients, which had curative potential in the patients with down staging. The median survival time was 11 months. Survival time of the 2 patients whose positive cytology became negative was 32 and 48 months, respectively (Table 3).

Table 3 : Clinical activity

Case	Level	RECIST	Down staging	Gastrectomy	Prognosis	Survival time (months)
1	1	PR	-	+	death	8
2	1	SD	-	+	death	10
3	1	SD	+*	+	alive	48
4	2	SD	-	+	death	17
5	2	PR	-	+	death	21
6	2	SD	-	-	death	15
7	2	SD	-	-	death	14
8	2	SD	-	+	death	10
9	2	SD	-	-	death	11
10	3	SD	+*	+	alive	32
11	3	SD	-	+	alive	30
12	3	SD	-	+	death	8
13	4	SD	-	+	death	9
14	4	SD	-	-	death	6
15	4	SD	-	+	death	5
16	5	PD	-	-	death	14
17	5	SD	-	-	death	7
18	5	SD	-	+	alive	11

* Positive adenocarcinoma cells in peritoneal cytology became negative.

DISCUSSION

Intraperitoneal infusion of PTX was generally well tolerated. The most frequently reported adverse event was Grade 3 leukocytopenia. DLT was leukocytopenia, MTD was 90 mg/m^2 and RD was 80 mg/m^2, respectively. These findings were supported by pharmacokinetics of PTX.

Because S-1 is the most widely accepted drug for gastric cancer in Japan, a lot of combination trials based on S-1 have been performed (8-10). Median overall survival was significantly longer in patients assigned to S-1 plus cisplatin (13.0 months) than in those assigned to S-1 alone (11.0 months) in the Phase III trial for advanced gastric cancer, however, peritoneal dissemination held 34%, 24% of each group, respectively (8). Significant differences in overall survival compared with S-1 alone revealed in any other regimens. It has not been established standard regimens for peritoneal metastasis of gastric cancer.

Intraperitoneal PTX in the phase II trial for the patients with small-volume residual carcinomas of the ovary, fallopian tube, or peritoneum was well tolerated, which included only moderate abdominal pain (grade 2 : 15.7%, grade 3 ; 1.3%) and minimal neutropenia (grade 2 ; 3.9% ; grade 3 ; 1.3%) (11). The incidence of Grade 3 neutropenia were observed in 32% of the patients with advanced gastric cancer in the treatment schedule comprised an intravenous infusion of 80 mg/m^2 PTX , repeated weekly three times for 4 weeks (12). These data suggested that intraperitoneal administration of PTX did not increase the incidence of drug-induced toxicities (13).

A pharmacokinetics study demonstrated that the PTX concentration in ascites remained in the range of the lower limit of cytotoxic effects and upper limit of blood system disorder from 4 to 72 hours after intravenous infusion of 60 and 80 mg/m^2 PTX. On the other hand, plasma concentrations of PTX were over upper limit of blood system disorder at 4 hours (14). In contrast, the PTX concentrations in 40, 60, 80 and 90 mg/m^2 cohort in this study remained in the optimal range. In 100 mg/m^2 cohort, the PTX concentrations were over upper limit of blood system. PTX concentrations in the ascites were approximately 1,000 folds higher than those in serum.

A major advantage after intraperitoneal delivery of PTX is high concentration in the peritoneal cavity (550-2,000 folds) compared with the systemic compartment (13). Drug exposure of high concentration is considered to have an advantage because antitumor effects increased dose dependent manner

as far as could be seen there were no severe toxicities in the experimental model (7).

Although this study is a phase I study, the response rate and survival could not be described exactly, two patients with positive adnocarcinoma cells and no macroscopically detected disseminate nodules had a long survival of over 30 months. The overall 5-year survival (43.8%) of advanced gastric cancer patients with intraperitoneal free cancer cells without overt peritoneal metastasis (CY+/P-) after extensive intraoperative peritoneal lavage followed by the intraperitoneal chemotherapy (EIPL-IPC: peritoneal lavage of 10 times using 1 L of physiological saline following cisplatin at a dose of 100 mg/body into the peritoneal cavity) was significantly better than that of the intraperitoneal chemotherapy (4.6%) and the surgery alone (0%) (15). It is important to detect positive adenocarcinoma cells in the peritoneal cavity to improve survival of the patients with peritoneal metastasis (16).

Concerning patients with macroscopically detected peritoneal metastasis, the utility of peritonectomy with chemohyperthermic peritoneal perfusion (CHPP) was reported, however, there are some problems regarding peritonectomy : complicated procedures and CHPP : severe stress to the patients and needs of specific and expensive instruments (17).

Fat solubility of PTX is suitable for intraperitoneal infusion, in contrast, Cremophor EL and ethanol is necessary as a solvent for clinical use, which causes acute hypersensitivity (18). For better and safe drug delivery system, various modifications of PTX have been developed and phase I trials were reported (19-21). Intraperitoneal PTX using the water-soluble solvent revealed excellent pharmacokinetics compared with Cremophor EL (22).

Intraperitoneal PTX including new modified drugs has high potentials to improve survival for the peritoneal metastasis of gastric cancer.

CONFLICT OF INTEREST STATEMENT

Mitsuo Shimada received a research grant from Research Support Foundation of the University of Tokushima and TAIHO Pharmaceutical. Co. Ltd. ; Other authors have no conflict of interest.

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