patients with advanced/recurrent gastric cancer was markedly longer than that in previous studies [2–12]. It was indicated that not only advances in first-line chemotherapy but also advances in second-line chemotherapy contributed to the prolongation of survival. However, no phase III study has verified the significance of second-line chemotherapy. The results of the Arbeitsgemeinschaft Internistische Onkologie (AIO) comparative study suggest its significance; this study was reported at the 2009 annual meeting of the American Society of Clinical Oncology (ASCO) [15]. In this study, patients in whom first-line therapy led to progressive disease were divided into 2 groups: best supportive care (BSC) and irinotecan groups, to evaluate the usefulness of irinotecan in second-line therapy. In regard to the statistical background, 60 patients per group (2 groups: 120 patients) were required, assuming that irinotecan administration may prolong the median survival time (MST) from 2.5 to 4 months, with an α error (paired) of 5% and a detection power of 80%. However, case registration was insufficient, and the clinical study was completed when 40 patients were enrolled in each of the two groups. The results of analysis were reported. In the irinotecan group, the response rate was 0%. However, the stable disease rate was 58%, and improvement of tumor-related symptoms was achieved in 44% of the patients. In addition, the MSTs in the irinotecan and BSC groups were 123 and 76 days, respectively. Statistically, the overall survival (OS) was longer in the irinotecan group ($p = 0.0027$). These results support the idea that second-line chemotherapy is appropriate in patients with a good general condition. However, which regimen is the most appropriate as second-line therapy must be investigated in the future.

Significance of second-line chemotherapy with respect to randomized comparative studies in Japan

The JCOG 9205 study was started by the Japan Clinical Oncology Group (JCOG) in 1992. Initially, 3 groups, 5-fluorouracil (5-FU), 5-FU + cisplatin, and uracil and tegafur (UFT) + mitomycin C (MMC) groups, were compared [4]. However, the mid-analysis results suggested that UFT + MMC therapy may be less potent than 5-FU therapy. After mid-analysis, the UFT + MMC group was excluded from the subject cohort. Finally, in this study, the results were compared between the 5-FU and 5-FU + cisplatin groups. The OS in the 5-FU + cisplatin group did not exceed that in the 5-FU group. The MST for monotherapy with 5-FU was 7.1 months, and the median progression-free survival (PFS) was 1.9 months. In the JCOG 9912 study, which was conducted subsequently, monotherapy with 5-FU was additionally employed as a control regimen [10]. The MST for monotherapy with 5-FU was 10.8 months, and the median PFS was 2.9 months. Survival data regarding monotherapy with 5-FU, involving different time-related background factors, were obtained in the two randomized comparative studies conducted by the same clinical study group (Table 1). The JCOG performed integral analysis regarding the two studies, focusing on second-line chemotherapy, and reported the results at the ASCO 2010 meeting [16]. To harmonize the inclusion criteria in the two studies, patients with intestinal stenosis in the JCOG 9205 study and those with adjuvant chemotherapy in the JCOG 9912 study were excluded. Overall survival, time to treatment failure (TTF), and OS minus TTF (OS–TTF) were compared after adjusting for baseline factors using the Cox proportional hazard model. Interestingly, the MST after second-line therapy in the 5-FU group was longer in the JCOG 9912 study.

There are two reasons for the above finding: firstly, the number of effective agents available for second-line therapy in the JCOG 9912 study was larger than the number available at the time of the JCOG 9205 study. In the JCOG 9205 study, early-generation drugs such as cisplatin and MMC were used for second-line therapy. On the other hand, in the JCOG 9912 study, newer drugs such as a taxane and irinotecan were primarily employed for second-line therapy; irinotecan- or taxane-containing regimens were selected in 9% (8/94) of the subjects in the JCOG 9205 study and in 67% (157/233) in the JCOG 9912 study. The difference in treatment options for second-line chemotherapy may have contributed to an MST difference of 3.7 months. On the other hand, the proportion of patients in the 5-FU group in whom treatment was switched to second-line chemotherapy should be compared between the two studies. In approximately 52% of patients receiving 5-FU alone in the JCOG 9205 study, treatment was switched to second-line chemotherapy. In the JCOG 9912 study, the percentage was approximately 83%, showing a 31% increase. This difference may also have led to the MST difference of 3.7 months. Even after adjusting for baseline factors, TTF was similar in the two studies; however, both OS and OS-TTF were longer in the JCOG 9912 study than in the JCOG 9205 study. It was concluded

Table 1 Differences of efficacy profiles and second-line treatment in the 5-fluorouracil arms between the JCOG 9205 and JCOG 9912 trials

	ORR (%)	PFS (months)	MST (months)	Second-line treatment (%)
JCOG 9205 trial [4]	9	1.9	7.1	52
JCOG 9912 trial [10]	11	2.9	10.8	83

JCOG Japan Clinical Oncology Group, *ORR* overall response rate, *PFS* progression-free survival, *MST* median survival time

that survival after treatment failure of 5-FU alone was longer in the JCOG 9912 study even when some potential confounding factors were adjusted for. The results of this combined analysis suggest that advances have been made in second-line chemotherapy and support the use of second-line chemotherapy for gastric cancer. Physicians likely play a key role in whether or not patients receive second-line chemotherapy. Unfortunately, we currently have little evidence to guide treatment. I recommend that patients and physicians earnestly discuss the risks and benefits of second-line chemotherapy using the current best evidence on tolerability and effectiveness.

Regional differences in second-line chemotherapy and new issues in global trials

Trials of the same regimen, S-1 plus cisplatin, were conducted in Japan and other countries. When comparing the SPIRITS trial (S-1 vs. S-1 plus cisplatin), which was carried out in Japan, with the FLAGS trial (5-FU plus cisplatin vs. S-1 plus cisplatin), which was conducted as a global study, there was a regional difference in second-line chemotherapy; there was a marked difference in the proportion of patients in whom treatment was switched to second-line chemotherapy between the two trials [9, 11]. The proportion of patients in whom treatment was switched to second-line chemotherapy was 73% in the SPIRITS study in Japan, whereas it was only 31% in the FLAGS trial. Such a low percentage was also common in other recently reported global studies. The second-line chemotherapy rates ranged from 70 to 83% in studies conducted in Japan, including the JCOG 9912 study [10–12], whereas the rate was only 15% in the REAL-2 trial involving the United Kingdom [7]. As a background factor, we must consider that the insurance coverage systems in Japan and other countries differ markedly. In particular, health insurance in the United Kingdom does not cover second-line chemotherapy; therefore, first-line chemotherapy is very important. The median survival in a phase III study recently reported in Japan was 2–3 months longer than that reported in Europe and the United States [7–12]. This finding may be associated with the difference in the proportion of patients in whom treatment was switched to second-line chemotherapy.

Currently, molecularly targeted agents for gastric cancer are being developed primarily in Japan and Korea and are being tested in global trials. As a new issue in these global trials, second-line chemotherapy has been emphasized. The ToGA study, in which Japanese and Korean patients accounted for more than 50% of the subjects, investigated the efficacy of first-line chemotherapy with trastuzumab in human epidermal growth factor receptor 2 (HER2)-positive advanced gastric cancer patients; 584 patients meeting eligibility criteria were randomly assigned to receive 5-FU or capecitabine + cisplatin (FC group: $n = 290$), or 5-FU or capecitabine + cisplatin + trastuzumab (FC + T group: $n = 294$) therapies. The median survival in the FC + T group (13.8 months) was significantly longer than that in the FC group (11.1 months) ($p = 0.0046$), suggesting the usefulness of trastuzumab in HER2-positive gastric cancer patients [17]. In this study, subset analysis showed regional differences in survival; trastuzumab did not influence survival in Asia, but markedly influenced survival in South America. This finding was possibly related to regional differences in second-line chemotherapy, as described above. Approximately 50% of the subjects consisted of Korean and Japanese patients. In these two countries, second-line chemotherapy is positively performed in clinical practice. On the other hand, in South America, second-line chemotherapy is rarely performed. Therefore, the influence of first-line chemotherapy; that is, that of trastuzumab, may have been more marked in South America.

Similarly, in the AVAGAST trial reported at the ASCO 2010 meeting, there were also differences in the proportions of patients in whom treatment was switched to second-line chemotherapy [18]. In that study, there was no influence of bevacizumab on survival in Asia, similar to the lack of influence of trastuzumab in the ToGA trial. In Pan-America, bevacizumab markedly influenced survival. This finding was possibly associated with regional differences in second-line chemotherapy (Table 2). In Asia, the proportion of patients in whom treatment was switched to second-line chemotherapy was high, 66%, whereas the values were 31 and 21% in Europe and Pan-America involving South America, respectively. Briefly, the influence of first-line chemotherapy on survival may be very marked in areas other than Asia. However, when many Japanese/Korean patients are registered, survival after second-line chemotherapy may be prolonged; therefore, there may be no significant difference in the OS. In the future, when developing molecularly targeted agents for first-line chemotherapy, we cannot ignore that there are regional differences in second-line chemotherapy. In planning global trials in the future, this issue should be sufficiently discussed.

Table 2 Proportions of patients receiving second-line chemotherapy by region in the AVAGAST trial [18]

Region	Patients entered (n)	Patients receiving second-line treatment (n)	%
Asia	376	248	66
Europe	249	78	31
Pan-America	149	32	21

Present status and future directions of second-line chemotherapy

Most recently reported studies of second-line chemotherapy consist of small-scale phase II or retrospective trials [19–33]. No randomized control trial to establish standard treatment has been conducted. In clinical practice, irinotecan, docetaxel, or paclitaxel is selected in most patients. However, the effects of monotherapy are limited [20–25]. Various combination therapies have been investigated in small-scale, phase II studies [26–33]. However, according to the results of some recent studies, the response rates ranged from approximately 10 to 20%, and PFS ranged from 2.5 to 4.0 months. There may be no marked differences among these combination therapies (Table 3). One study reported a median survival of 12 months. However, this may have depended on patient selection. As of now, that is all the information we can share. At the time of this writing, I think monotherapy is a reasonable option as a second-line treatment, and combination strategies should be used as a fall-back position. In Japan, weekly paclitaxel is widely used as the second-line chemotherapy in daily clinical practice. On the other hand, the AIO comparative study supported the use of irinotecan for second-line chemotherapy [15]. Much debate has focused on whether irinotecan or weekly paclitaxel is the better second-line agent. Among randomized control trials of second-line chemotherapy that are being conducted, "a randomized phase III study of irinotecan versus weekly paclitaxel in unresectable or recurrent gastric cancer refractory to combination therapy of fluorouracil plus platinum (WJOG 4007G)", has been carried out by the West Japan Oncology Group (WJOG). In this study, the primary endpoint was overall survival. Secondary endpoints were PFS, adverse events, and the response rate in patients with target lesions. The sample size was 220 in total, which allowed for the detection of irinotecan superiority over weekly paclitaxel in terms of OS. Final analysis will be performed in 2011. These study results are very important. It should be clarified which of the two agents, irinotecan or paclitaxel, is appropriate as a biologic, platform agent for second-line chemotherapy, and whether the effects of the two agents are similar.

Currently, several second-line or subsequent molecularly targeted agents are being developed and tested in global studies (Table 4). A randomized control trial of lapatinib involving HER2-positive gastric cancer patients (TYTAN trial) is being conducted (weekly paclitaxel vs. weekly paclitaxel + lapatinib). Furthermore, a randomized control trial of a mammalian target of rapamycin (mTOR) inhibitor, everolimus, for BSC is being performed in patients receiving second- and third-line therapies (GRANITE-1 trial) [34]. For new drug development, global trials are also necessary in the future. However, in randomized control trials in which OS is established as the primary endpoint of first-line chemotherapy, it is difficult to detect a difference unless molecularly targeted agents with a clear target, such as trastuzumab, are employed; this difficulty arises because there are regional differences in second-line chemotherapy. In particular, Japan and Korea,

Table 3 Efficacy profiles of combination chemotherapy in the second-line setting

Regimen	ORR (%)	PFS (months)	MST (months)	Reference number
Paclitaxel/doxifluridine	18.2	4.0	10.7	[26]
Paclitaxel/capecitabine	34.6	4.5	7.5	[27]
Docetaxel/doxifluridine	18.8	2.6	12.7	[28]
Docetaxel/irinotecan	20.4	2.7	8.9	[29]
Docetaxel/oxaliplatin	10.5	4.0	8.1	[30]
Irinotecan/5-fluorouracil	18.2	2.3	5.1	[31]
Irinotecan/capecitabine	17.0	3.1	6.5	[32]
Methotrexate/5-fluorouracil	9.0	NE	7.9	[33]

ORR overall response rate, *PFS* progression-free survival, *MST* median survival time, *NE* not evaluated

Table 4 Phase III studies of targeted agents for second-line treatment in advanced gastric cancer

Agent	Target	Chemotherapy partner	*N*	Endpoint	Status
Lapatinib	HER2 EGFR	Paclitaxel	260	OS	Ongoing
Ramucirumab	VEGFR-2	Paclitaxel	663	OS	Ongoing
Everolimus	mTOR	None	442	OS	Ongoing

OS overall survival, *mTOR* mammalian target of rapamycin, *HER2* human epidermal growth factor receptor 2, *EGFR* epidermal growth factor receptor, *VEGFR-2* vascular endothelial growth factor receptor 2

where second-line chemotherapy is actively performed, play a principal role in registration. For the future development of molecularly targeted agents, it might be necessary to discuss the adoption of PFS as the primary endpoint.

Conclusions

At this time, no standard second-line chemotherapy has clearly emerged in gastric cancer treatment, and none of the new molecularly targeted agents under investigation has been identified as being appreciably useful for second-line-chemotherapy. Given the lack of solid evidence, it is too early to know whether a number of novel regimens will ultimately achieve traction as useful standard second-line chemotherapies. New evidence and new drugs are needed to make the necessary further improvements in the management of gastric cancer. In global trials, however, we have learned of the difficulties in selecting survival benefit as the primary endpoint, with these difficulties arising because of the regional differences in the management of this disease. In planning global trials, this new issue should be sufficiently discussed.

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Application of electrolysis for detoxification of an antineoplastic in urine

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ABSTRACT

Antineoplastics in excreta from patients have been considered to be one of the origins of cytotoxic, carcinogenic, teratogenic, and mutagenic contaminants in surface water. Recent studies have demonstrated that antineoplastics in clinical wastewater can be detoxified by electrolysis. In this study, to develop a method for the detoxification of antineoplastics in excreta, methotrexate solution in the presence of human urine was electrolyzed and evaluated. We found that urine inhibits detoxification by electrolysis; however, this inhibition decreased by diluting urine. In urine samples, the concentrations of active chlorine generated by anodic oxidation from 0.9% NaCl solution for inactivation of antineoplastics increased in dilution-dependent and time-dependent manner. These results indicate that electrolysis with platinum-based iridium oxide composite electrode is a possible method for the detoxification of a certain antineoplastic in urine.

1. Introduction

The contamination of surface water by pharmaceutically active compounds (PhACs) including their metabolites has been detected by the advances in the measurement or the detection. PhACs include antibiotics (Hirsch et al., 1999), antiepileptic drugs, lipid regulators, antiphlogistics, beta-blockers, iodinated X-ray contrast media, and estrogen (Holm et al., 1995; Ternes, 1998; Kolpin et al., 2002; Heberer, 2002; Seino et al., 2004). These PhACs are not eliminated completely in municipal sewage treatment plants (Heberer, 2002). It is suggested that trace amounts of antineoplastics bring irreversible and cumulative effect on environmental organisms without a threshold, and affect human health directly or indirectly via the ecological system (Daughton and Ternes, 1999; Eitel et al., 2000). The antineoplastics in effluent originate from pharmaceutical plants, hospitals, and patients' excreta (Jφrgensen and Halling-Sφrensen, 2000; Kümmerer, 2001; Heberer, 2002). The occurrence and fate of antineoplastics in environment are emerging issues, because the substances have carcinogenic, teratogenic, and mutagenic properties (Skov et al., 1990), and reveal low biodegradability (Kümmerer and Al-Ahmad, 1997; Kümmerer et al., 1997; Steger-Hartmann et al., 1997; Al-Ahmad and Kümmerer, 2001).

In pharmaceutical plants, toxic substances are well controlled by chemical treatment, rinsing, and heating/incineration (Vaccari et al., 1984; Castegnaro et al., 1997). Since the National Institutes of Health of USA recommended the incineration for antineoplastics in its guidelines (Vaccari et al., 1984), antineoplastics in clinical wastewater have been incinerated in many countries (Eitel et al., 2000). The wastewaters containing antineoplastics from plants and hospitals was disposed with untreated form, but in recent several years, some of wastes have been collected and treated by incineration. Because incineration consumes huge amount of energy and generates large amount of greenhouse gas, incineration produces new environmental load. Further, in municipal sewage treatment plants it is difficult to decompose contaminating antineoplastics in excreta from patients treated with antineoplastics (Jφrgensen and Halling-Sφrensen, 2000; Heberer, 2002).

Recently, electrolysis with platinum-based iridium oxide composite electrode of clinical wastewater containing antineoplastics has been demonstrated to be effective for decreasing their toxicity (Hirose et al., 2005). This method mainly involves the

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degradation by oxidants such as hypochlorite generated from anodic oxidation in NaCl solution. On the basis of the findings, an apparatus for detoxification of clinical wastewater was designed and its performance was evaluated (Kobayashi et al., 2008). Our group has demonstrated that the electrolysis method decomposes antineoplastics in wastewater; nevertheless, it has not been confirmed whether electrolysis decomposes antineoplastics in excreta. In the present study, we attempted to clarify whether electrolysis decreases cytotoxicity and concentration of an antineoplastic in the presence of urine.

2. Materials and methods

2.1. Reagents and sample preparation

One of antineoplastics, methotrexate (MTX), was purchased from Calbiochem Co. (San Diego, USA). MTX structure was illustrated in Fig. 1. Fresh urine used for the experiments was collected from a healthy adult male. Cl^- concentration in urine of this male was 89–171 mEq/L, measured by the ion-selective electrode method. The collected urine was diluted with distilled water and MTX was added at a final concentration of 880.2 μM. To enhance the generation of active chlorine and to minimize the osmotic pressure on cells in cytotoxicity assay, 2.25 g of NaCl was added to 250 mL (i.e., 0.9% NaCl=153 mM) mixture, and the mixture served as an experimental sample. The conductivity in the presence of urine sample was equivalent to 0.9% NaCl solution. The samples were electrolyzed and analyzed for the quantity and cytotoxicity of MTX. In some experiments, active chlorine concentration was examined in the diluted urine following electrolysis.

2.2. Measurement of active chlorine

Active chlorine was measured using equipment (RC-2Z; Kasahara Chemical Instruments Co. Ltd., Saitama, Japan) based on absorbance method. This equipment measures hypochlorite and hypochlorous acid ions produced by anodic oxidation from 0.9% NaCl solution as active chlorine.

2.3. Electrolysis procedure and neutralization of hypochlorite

A sample of 250 mL was electrolyzed in a 300 mL glass beaker using a pair of platinum-based iridium oxide composite electrode (115 mm × 35 mm, placed 5 mm apart) for a designated time at a constant current of 1 A, and voltage is from 3.5 V to 4.0 V. Electrode is purchased from Japan Carlit Co. Ltd. (Tokyo, Japan). To neutralize cytotoxic hypochlorite, 30 μL of 20% (w/v) sodium thiosulfate was added to 210 μL of an electrolyzed sample. After electrolysis, samples were stocked in a freezer at −20 °C until measurement.

2.4. Absorption spectra of diluted urine

Urine samples before and after electrolysis were diluted 50 fold with distilled water, and the absorption spectra of diluted samples were measured with UV–vis spectophotometer (UV-160A, Shimadzu Seisakusyo, Kyoto, Japan) at the wavelength between 200 and 800 nm.

2.5. Immunoassay

MTX concentrations were measured by the fluorescence polarization immunoassay (FPIA) method using a commercially available kit (TDx-Methotrexate II Dynapack; Abbott Japan Co. Ltd., Matsudo, Japan), in accordance with the manufacturer's instructions. The calibration curves were determined using 0.08802–880.2 μM MTX in various solvents, 0.9% NaCl solution, and 2-fold-diluted urine with/without electrolysis.

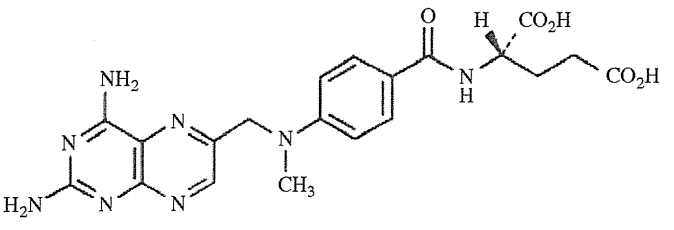

Fig. 1. Structure of methotrexate.

2.6. Evaluation of cytotoxicity

The cytotoxicity was evaluated using the Molt-4 cell line, which is a human lymphoblastoid cell line. A sample was diluted 4-fold with RPMI-1640 medium containing 10% fetal bovine serum, mixed with the same volume of medium containing 5×10^5 cells/mL, and cultured in a U-bottom 96-well microplate in 5% CO_2 atmosphere at 37 °C for 3 days. One hundred microliters of the cell culture was transferred to a flat-bottom 96-well microplate, mixed with 10 μL of the solution included in the WST-8 cell counting kit (Dojin, Kumamoto, Japan), and incubated at 37 °C for 1 h. The optical density of the plate was measured using an optical densitometer (ImmunoMini NJ-2300, Microtec Co. Ltd., Tokyo, Japan) at a wavelength of 450 nm with a reference wavelength of 620 nm. The survival rate of Molt-4 cells was defined as the absorbance ratio of the well with a sample to that without a sample. The 50% cytotoxic concentration (CC_{50}), which is the 50% survival rate of Molt-4 cells, was calculated as an index of cytotoxicity of a sample.

3. Results

3.1. Active chlorine generation in the presence of urine

To examine whether the electrolysis in the presence of urine generates active chlorine, the concentration of active chlorine in electrolyzed urine was measured and compared with that in 0.9% NaCl solution (Fig. 2a). In 0.9% NaCl solution, the concentration of active chlorine increased in the first two hours of electrolysis, and then reached a plateau at approximately 3000 mg/L. In 2-fold, 4-fold, and 8-fold-diluted urine added to same amount of NaCl, the concentration of active chlorine slightly increased to 168.33 ± 18.93 mg/L in 2-fold-diluted urine or 345.00 ± 5.00 mg/L

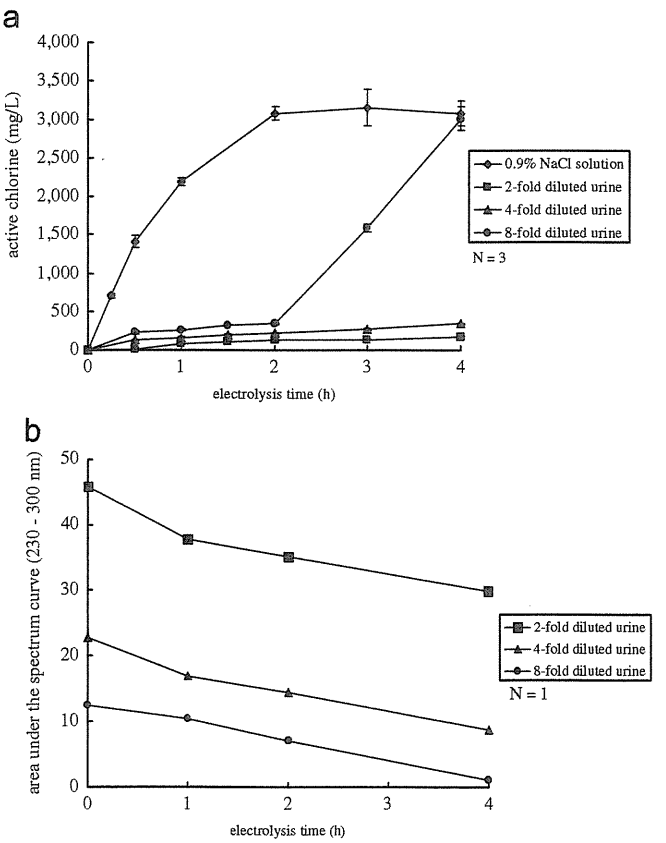

Fig. 2. Generation of active chlorine in the presence of urine (a) and the area under the spectrum curve between 230 and 300 nm of diluted urine (b) during electrolysis. Concentrations of active chlorine after electrolysis in the presence of one-half, one-fourth, and one-eighth part volumes of urine were compared with that after electrolysis of saline (a). The absorbance of each diluted urine was measured before electrolysis and 1 h, 2 h and 4 h of electrolysis, respectively (b). The area under spectrum curve between 230 and 300 nm was calculated, and the amount of urinary content was represented as the index.

in 4-fold-diluted urine after 4 h of electrolysis. In 8-fold-diluted urine, the concentration of active chlorine also slightly increased after electrolysis for 2 h, and increased to 3010.00 ± 155.56 mg/L after 4 h of electrolysis. These results indicate that the generated active chlorine immediately reacted with urinary substances and consumed. We measured the absorption spectra of urine samples before and after electrolysis to estimate the amount of urinary substances decomposed by electrolysis. The absorption spectrum between 200 and 800 nm was measured, and substances at the absorption between 230 and 300 nm was detected. The area under the spectrum curve between 230 and 300 nm of each diluted urine, which is the amount of the measurable urinary substances, was decreased during the electrolysis. The amounts of urinary substances in 2-fold, 4-fold, and 8-fold-diluted urine before electrolysis were 45.82, 22.84, and 12.48, respectively. The same indices were decreased in time-dependent manners, and after 4 h of electrolysis, the indices of 2-fold, 4-fold, and 8-fold-diluted urine were 29.89, 8.81, and 1.13, respectively (Fig. 2b). According to Fig. 2a, in 8-fold diluted urine sample, active chlorine generated effectively after 2 h of electrolysis, and the amount of urinary substances at that time is 7.02 in the area under the spectrum curve index. When the amount of urinary substances was decreased under the threshold level, active chlorine was considered to be effectively generated.

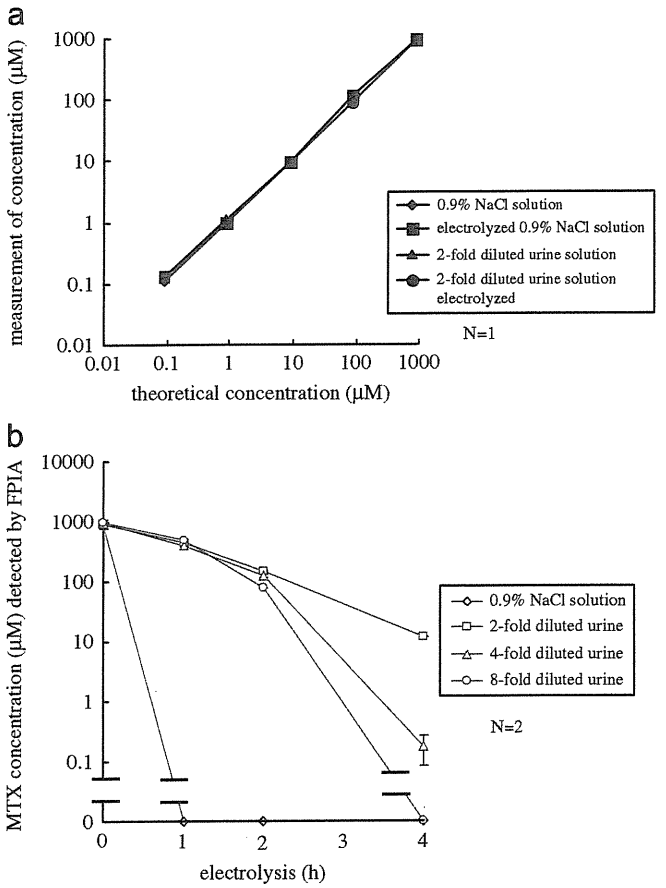

Fig. 3. Generation of calibration curves for MTX concentration determined by the FPIA method (a) and the destruction of MTX by electrolysis in the presence of urine (b). MTX (880.2 μM) was diluted with 0.9% NaCl solution, electrolyzed 0.9% NaCl solution, 2-fold-diluted urine, and 2-fold-diluted urine electrolyzed 10-fold, and each of the calibration curves was shown (a). It is confirmed that FPIA method is consistent with different diluent used, therefore, MTX concentrations after electrolysis in the presence of half, quarter, or one-eighth part volume urine were measured by the FPIA method, and compared with those in the absence of urine and with unelectrolyzed sample (b).

3.2. Inhibition of destruction of MTX by urine

MTX concentration was measured by the FPIA kit. Because the kit is originally designed to measure MTX concentration in plasma or serum, we first confirmed whether it accurately determines the concentration of MTX in our sample. MTX at a concentration of 880.2 μM was prepared in 0.9% NaCl solution, electrolyzed 0.9% NaCl solution mixed with sodium thiosulfate, 2-fold-diluted urine solution, and electrolyzed 2-fold-diluted urine solution mixed with sodium thiosulfate. Then, these MTX solutions were serially diluted 10 fold with the corresponding solvents up to 0.08802 μM. The concentration of MTX was determined and calibration curves were generated (Fig. 3a). The coefficient values (R^2) were over 0.998 for all solvents. Fig. 3a illustrates that MTX concentration was accurately determined by the kit under these conditions. To examine whether urine inhibits the degradation of MTX by electrolysis, and whether the inhibition decreased by dilution of samples, MTX solution was electrolyzed in the presence of urine (Fig. 3b). In each solvent, MTX concentration decreased during electrolysis in time-dependent manners, and in 0.9% NaCl solution, MTX concentration was below detection limit at 1 h of electrolysis. After 4 h electrolysis of MTX solution with one-half and one-fourth part volumes of urine, the MTX concentration decreased to 11.8 ± 0 μM and 0.175 ± 0.09 μM, respectively. In the presence of one-eighth part volume of urine, the 4 h electrolysis decreased MTX concentration to below the detection limit of the assay.

3.3. Cytotoxicity of electrolyzed urine containing MTX

MTX was confirmed to be effectively decomposed when urine samples were 8-fold diluted and electrolyzed for 4 h. To evaluate whether MTX was detoxified by electrolysis under this condition, MTX solution was electrolyzed in the presence of urine. In the presence of one-half or one-fourth part of the volume of urine, CC_{50} slightly increased to 1.993 ± 0.04 μM or 11.173 ± 8.44 μM after 4 h of electrolysis, respectively. In the presence of one-eighth part volume of urine, CC_{50} reached the detection limit of the assay after 4 h of electrolysis, which is the same as in 0.9% NaCl solution (Table 1).

Table 1
50% cytotoxic concentrations (CC_{50}) of MTX before and after electrolysis.

Conditions	Solvent/CC50 (μM)			
	0.9% NaCl solution	2-fold-diluted urine	4-fold-diluted urine	8-fold-diluted urine
Before electrolysis	0.104 ± 0.009	0.079 ± 0.007	0.085 ± 0.014	0.034 ± 0.001
4 h of electrolysis	106.333 ± 7.72	1.993 ± 0.04	11.173 ± 8.44	142.327 ± 24.56

N=3.

4. Discussion

This study was performed to clarify the possibility of detoxification of antineoplastics in excreta by electrolysis. We confirmed that electrolysis generates active chlorine and decomposes MTX in the presence of urine. Although urinary contents consumed active chlorine and resulted inhibition of degradation and detoxification of MTX, the inhibition was avoided by diluting urine. Cytotoxicity in electrolysis samples was not detected, which indicated that there was no new cytotoxicity for human cells with the electrolysis.

Antineoplastics are excreted as either detoxified or toxic form after administration. Some kinds of antineoplastics with their toxicity remaining are excreted as unchanged form. If these antineoplastics are not treated efficiently, environmental load to surface water is more serious than those, which are detoxified by metabolism before excretion. Because MTX is excreted in unchanged form (Zurek et al., 1968), we selected this chemical in this study. In clinical use, when MTX at a high dosage is administered (100–350 mg/kg body weight), it is reported that the average serum MTX concentration is above 100 μM after 6 h, and less than 0.1 μM after 72 h (Takada et al., 1980). Approximately 90% of MTX is excreted in an unchanged form in urine within 24 h when administered at a high dosage (Zurek et al., 1968). In this study, MTX was prepared at 880.2 μM, and the concentration is considered as practical when the method is applied to the clinical settings. Under these considerations of the disposition of antineoplastics, MTX is considered as one of the most suitable drugs to demonstrate whether electrolysis is the possible method for detoxification of antineoplastics in excreta.

In the previous studies, Hirose et al. (2005) demonstrated that electrolysis detoxified various categories of antineoplastics and their mixture experimentally, and Kobayashi et al. (2008) successfully applied the method to clinical wastewater. Those previous studies and the present study indicated that electrolysis is a possible method for the detoxification of antineoplastics in urine.

Platinum-based iridium oxide composite electrode is employed in this study. Because this electrode is advantageous for generating active chlorine, is good for conductivity, and is stable for chemicals even at high voltage, we have evaluated its performance (Hirose et al., 2005; Kobayashi et al., 2008). Recently, coating materials to cover the surface of electrodes has been investigated, and synthetic boron-doped diamond thin film (BDD) has recently evaluated for its efficacy (Panizza and Cerisola, 2009). Nevertheless, among materials of electrode, the main principle is the same, which is, electrochemical degradation that electrode triggers anodic oxidation (Brillas et al., 2005; Sirés et al., 2006; Zhao et al., 2009; Murugananthan et al., 2010). In this study, electrolysis conditions including the material of electrode may not be optimized; however, our result confirmed that an antineoplastic is able to degrade effectively in the presence of urine with platinum-based iridium oxide composite electrode. Therefore, this study provides important information with regard to electrochemical degradation of PhACs by electrolysis.

To prevent contamination by PhACs in effluent, municipal sewage treatment plants are equipped with various devices (Andreozzi et al., 2003; Ternes et al., 2003; Salter et al., 2011). However, no appropriate method for the detoxification of antineoplastics contained in excreta is available at present. Further experiments that directly show the detoxification of other antineoplastics and their metabolites by electrolysis are required on the basis of this study.

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ORIGINAL ARTICLE

A phase II study of biweekly mitomycin C and irinotecan combination therapy in patients with fluoropyrimidine-resistant advanced gastric cancer: a report from the Gastrointestinal Oncology Group of the Japan Clinical Oncology Group (JCOG0109-DI Trial)

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Abstract
Background Preclinical studies have shown that mitomycin C (MMC) acts synergistically with irinotecan (CPT-11). In this phase II study, we evaluated the efficacy and toxicity of MMC/CPT-11 therapy as second-line chemotherapy for patients with fluoropyrimidine-resistant advanced gastric cancer.
Methods Eligible patients had evidence of tumor progression despite prior treatment with fluoropyrimidine-based regimens or had relapsed within 6 months after completion of therapy with adjuvant fluoropyrimidines. Treatment consisted of MMC (5 mg/m^2) and CPT-11 (150 mg/m^2) administered i.v. every 2 weeks. The primary endpoint was the response rate (RR). Our hypothesis was that this combination therapy was efficacious when the lower boundary of the 95% confidence interval (CI) of the RR exceeded 20% of the threshold RR.

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Results Between April 2002 and July 2003, 45 eligible patients were registered and analyzed. Among the 45 patients, 40 (89%) had previously received chemotherapy for metastasis and 24 (53%) had a performance status (PS) of 0. Thirteen partial responses were obtained among the 45 patients, resulting in an overall RR of 29% (95% CI, 16–42%). The median time to progression was 4.1 months, and the median survival time was 10 months, with a 1-year survival rate of 36%. Grade 4 neutropenia was observed in 29% of the patients, whereas febrile neutropenia occurred in 9%. The incidence rates of grade 3 nausea and diarrhea were 13 and 2%, respectively.

Conclusions Although this study did not achieve the per-protocol definition of activity, the progression-free survival and overall survival appeared to be promising, with acceptable tolerability. Thus, MMC/CPT-11 therapy as second-line chemotherapy for fluoropyrimidine-resistant advanced gastric cancer presents a potential treatment option in patients with a good PS.

Keywords Gastric cancer · Mitomycin-C · Irinotecan · Fluoropyrimidine-resistant · Second-line chemotherapy

Introduction

Gastric cancer is the most common malignancy in Asian countries, with approximately 50,000 deaths in Japan annually [1]. The treatment of choice for this malignancy is primary tumor resection. In patients with curatively resected stage I–III gastric cancer, the 5-year survival proportion is >50%; however, this proportion remains at <10% in stage IV or recurrent disease. Randomized trials have demonstrated that fluorouracil-based regimens improve survival proportions in patients with advanced gastric cancer (AGC) compared with best supportive care (BSC) alone as first-line chemotherapy [2–4]. Moreover, combination chemotherapy results in superior outcomes compared with monotherapy. In Japan, the efficacy and toxicity of the combination of an oral fluoropyrimidine (S-1) and platinum was previously evaluated in the phase III SPIRITS (S-1 plus cisplatin vs. S-1 alone for first-line treatment of AGC) trial. S-1 plus cisplatin resulted in superior overall survival (OS) compared with S-1 alone [hazard ratio (HR), 0.77; 95% confidence interval (CI), 0.61–0.98%; $P = 0.04$], with an impressive median OS of 13.0 months [5]. The Japan Clinical Oncology Group (JCOG) 9912 trial (5-fluorouracil [FU] alone vs. S-1 alone vs. irinotecan [CPT-11] plus cisplatin [CDDP] combination for the first-line treatment of AGC) was also conducted in Japan. S-1 showed significant noninferiority for progression-free survival (PFS) and OS compared with 5-FU alone; however, CPT-11 plus CDDP showed no significant superior effects on PFS and OS compared with 5-FU alone [6]. In Japan, S-1 plus CDDP combination therapy is considered the standard first-line treatment for AGC.

Thuss-Patience et al. [7] reported at the 2009 Annual Meeting of the American Society of Clinical Oncology (ASCO) that CPT-11 monotherapy significantly prolonged OS compared with BSC as second-line chemotherapy. Although that report was the first randomized phase III study investigating second-line chemotherapy for AGC, no objective responses were observed. Thus, a consensus regarding the standard regimen for second-line chemotherapy has not yet been obtained.

Many AGC patients who failed to respond to first-line chemotherapy showed symptoms of pain, weight loss, or nausea due to their progressive disease. Thus, the induction of a tumor response is as important as delaying tumor progression for as long as possible. Patients who received combination chemotherapy showed higher response rates than those who received single-agent chemotherapy alone. Therefore, combination chemotherapy is preferable to single-agent chemotherapy for palliation. Moreover, combination chemotherapy may prolong OS compared with single-agent chemotherapy alone.

CPT-11 is a potent topoisomerase I inhibitor and is effective against AGC. In a phase II trial, the response rate (RR) to CPT-11 alone was 16% in previously treated AGC patients [8]. The administration of a CDDP and CPT-11 combination in AGC patients resulted in a higher RR and longer time to progression (TTP) [9–11]. As mentioned above, CDDP/CPT-11 did not significantly prolong OS over 5-FU, but induced a significantly higher RR than 5-FU in the JCOG9912 trial [6]. A 5-FU, leucovorin (LV), and CPT-11 combination produced a higher RR and longer TTP than CDDP/CPT-11 in AGC patients [12]. In another randomized phase III trial, 5-FU/LV/CPT-11 showed a trend to have superiority in TTP over CDDP/5-FU (5.0 vs. 4.2 months, respectively; HR, 1.23; 95% CI, 0.97–1.57%; $P = 0.088$), and a better safety profile [13]. These results support the finding that CPT-11 is active against AGC.

Mitomycin C (MMC) is also effective against AGC. Preclinical studies have shown that a MMC and CPT-11 combination synergistically inhibits tumor growth in vitro [14]. This is due to the possible induction of topoisomerase I gene expression by MMC, thereby increasing tumor cell sensitivity to CPT-11. A phase I/II study of this combination recommended an MMC dose of 5 mg/m^2 and a CPT-11 dose of 150 mg/m^2 administered biweekly [15]. The dose-limiting toxicities of this combination regimen when administered at 10 mg/m^2 for MMC and 150 mg/m^2 for CPT-11 were grade 4 neutropenia with or without febrile neutropenia and grade 3 diarrhea. The overall RR was 50% (15/30 patients), and 5 of 14 patients (36%) with prior chemotherapy showed a partial response (PR). We

previously showed that MMC and CPT-11 combination chemotherapy was effective and well tolerated in patients with fluoropyrimidine-resistant metastatic colorectal cancer; the RR, median TTP, and median survival time (MST) were 34% (95% CI, 20–49%), 4.2 months, and 11.9 months [16], respectively.

These results led us to conduct the present phase II clinical trial to investigate the efficacy and toxicity of MMC/CPT-11 therapy in patients with AGC resistant to a fluoropyrimidine-containing regimen in the JCOG0109-DI study.

Patients and methods

Eligibility

A patient was considered eligible if there was evidence of a refractory response to one prior chemotherapy containing fluoropyrimidine, which was any of the following types of history of chemotherapy:

1. In the case of unresectable gastric cancer, disease progression detected while receiving front-line chemotherapy containing fluoropyrimidine, or confirmed immediately after the discontinuation for any reason other than disease progression.
2. In the case of recurrent gastric cancer, recurrence detected within 24 weeks from the last dose of postoperative adjuvant chemotherapy containing fluoropyrimidine, and further chemotherapy was not administered after recurrence.
3. In the case of recurrent gastric cancer detected 25 weeks after the last dose of postoperative adjuvant chemotherapy, disease progression detected while receiving front-line chemotherapy containing fluoropyrimidine after recurrence, or confirmed immediately after the discontinuation for any reason other than progression.
4. In the case of recurrent gastric cancer treated with neoadjuvant chemotherapy, the effect of neoadjuvant chemotherapy containing fluoropyrimidine was stable disease, progressive disease, or not evaluated, and recurrence was identified after curative resection. Chemotherapy was not performed following recurrence.
5. In the case of recurrent gastric cancer treated with neoadjuvant chemotherapy, the chemotherapy effect was a complete response or PR, and progression was detected during one chemotherapy containing fluoropyrimidine after recurrence, or confirmed immediately after discontinuation for any reason other than progression.

Disease progression and the nonefficacy of neoadjuvant chemotherapy were believed to represent clinical failure by treating physicians. Elevation of the level of a tumor marker, such as carcinoembryonic antigen (CEA), was not accepted as adequate evidence for treatment failure. Documentation of evidence of a refractory response by computed tomography (CT) and magnetic resonance imaging was required.

For the other eligibility criteria, patients must be between 20 and 75 years of age, and have an Eastern Cooperative Oncology Group performance status (PS) of 0 to 2, adequate baseline bone marrow function [white blood cell (WBC) and platelet counts $\geq$4,000 and 100,000/mm^3, respectively], adequate hepatic function (serum bilirubin level $\leq$1.5 mg/dl and both serum aspartate aminotransferase and alanine aminotransferase levels $\leq$100 U/l), adequate renal function (serum creatinine level $\leq$1.5 mg/dl), adequate respiratory function (arterial partial pressure of oxygen $\geq$70 mmHg), and have received no blood transfusion within 14 days before enrollment. All patients were required to have $\geq$1 measurable lesion according to the Response Evaluation Criteria in Solid Tumors (RECIST).

Patients were excluded if they had symptomatic brain metastasis, symptomatic ascites and/or pleural effusion, previous history of MMC or CPT-11 chemotherapy, pre-existing diarrhea of >4 times/day, suspicion of existing active bleeding which needed blood transfusion at 14 days prior to registration in this study, or a high risk of a poor outcome due to concomitant nonmalignant disease (i.e., cardiac, pulmonary, renal, or hepatic disease; poorly controlled diabetes; or uncontrolled infection), or severe psychiatric disease. Pregnant or lactating women were excluded.

The study protocol was approved by the JCOG Clinical Trial Review Committee and the institutional review board of each participating hospital. All patients gave their written informed consent.

Treatment plan

The treatment schedule consisted of one MMC dose (5 mg/m^2, bolus injection), then CPT-11 (150 mg/m^2, 90-min i.v. infusion) repeated every 2 weeks, as described previously [16]. All patients were treated on an outpatient basis and were recommended to receive both a 5-hydroxytryptamine-3-receptor antagonist and dexamethasone to prevent emesis. Subsequent treatment cycles were withheld until the WBC and platelet counts were $\geq$3,000 and 100,000/mm^3, respectively; diarrhea was $\leq$grade 1; and there were no infection symptoms such as pyrexia ($\geq$38°C). When the treatment course was delayed within 8 days from the planned schedule, the same dosage levels as those used previously were administered. When the treatment course was delayed beyond 8 days and within 21 days from the planned schedule, one lower dose level (CPT-11 level -1,

125 mg/m^2; level -2, 100 mg/m^2) than the previous level was administered, while the MMC dose was maintained at 5 mg/m^2. The treatment course was discontinued if it could not be started within 21 days from the planned schedule. When grade 4 leukopenia or thrombocytopenia occurred in a previous treatment course causing a delay within 8 days, the same dosage levels as those used previously were administered. When grade 2 diarrhea or higher was observed in a preceding course, dosages 1 level lower than the previous dosages were administered.

Treatment was repeated until disease progression or when severe toxicity was observed. The total MMC dose was limited to 50 mg/m^2, to prevent cumulative toxicity (e.g., interstitial pneumonia and hemolytic uremic syndrome), and thereafter CPT-11 alone was administered. This indicates that the maximum number of total treatment cycles of MMC/CPT-11 therapy is 10 cycles.

Evaluation of response and toxicity

During protocol treatment, the patient's signs and symptoms, as well as laboratory data (i.e., WBC with differential counts, liver function tests, urea nitrogen, creatinine, electrolytes, and urinalysis) were examined biweekly. Adverse events were evaluated using the National Cancer Institute-Common Toxicity Criteria version 2.0. Tumor response was assessed by CT every 4 weeks. The response of measurable and evaluable disease sites was assessed by each investigator in accordance with RECIST, and then reviewed by central review at the group meeting.

Statistical analysis

For this study, the primary endpoint was the RR and the secondary endpoints were OS and toxicity. Here, we used the standard design (attained design) of the Southwest Oncology Group [17]. Based on reports of RRs of 22% with paclitaxel alone [18] and 16% with CPT-11 alone [8] in the second-line setting and an RR of 36% in phase I/II studies of MMC/CPT-11 therapy [15], the RR in this study was expected to be within 30–40% for a future phase III trial. Here, the required sample size was calculated to be 45 patients, with the following parameters: $\alpha = 0.05$, $\beta = 0.10$, threshold response rate $(p_0) = 20\%$, and expected response rate $(p_a) = 40\%$. Interim analysis was performed when the number of enrolled subjects reached 25. The significance level for the interim analysis was set as $P < 0.02$. Furthermore, when the number of patients who reached RR was <5 at the interim analysis, the study was prematurely discontinued because it would have been difficult to exceed the expected RR despite further patient accumulation, or because it would not be worth advancing this regimen to an ensuing clinical study. When the study was not completed after the interim analysis, the number of patients was increased to 45 in order to allow the null hypothesis (threshold RR) to be tested. When α was <0.05, or when the lower boundary of the 95% CI of the RR exceeded 20% of the threshold RR, this therapy was considered to be efficacious as chemotherapy for gastric cancer patients who had received pretreatment. That is, when ≥16 of 45 patients had a PR, this study was judged to be positive. Here, patient enrollment was not temporarily discontinued.

OS was defined as the time from the registration date to death as a result of any cause. PFS was defined as the time from the registration date to the first documentation of objective tumor progression. Time-to-event and OS data were summarized using the Kaplan–Meier method.

Results

Patient population and study treatment

Between April 2002 and July 2003, 45 patients (33 men, 12 women) from 12 hospitals were enrolled and analyzed. Table 1 shows the demographic data, baseline disease, and regimens of prior chemotherapy. The median age was 64 years (range 36–75), and all patients had a good PS of 0 or 1. Eighteen patients (40%) had diffuse-type gastric cancer. As for prior chemotherapy, 40 (89%) had previously received chemotherapy for metastasis, whereas 5 had received adjuvant chemotherapy. In the first-line chemotherapy, 33 patients (73%) had received 5-FU or S-1 alone.

In all 45 patients, MMC/CPT-11 therapy was administered 281 times, and the median number of doses was 6 (range 1–10). Of the 45 patients, 10 (22%) completed the planned 10 chemotherapy cycles. In the remaining 35 patients, the reasons for treatment discontinuation were disease progression in 25, toxicity in 6, patient's refusal in 3, and death in 1. Regarding CPT-11 administration, 11 patients (24%) required -1 level dose reduction and 8 (18%) required -2 level reduction because of leukopenia and thrombocytopenia.

Efficacy

Of the 45 patients, 13 showed a PR (RR: 28.9%; 95% CI, 15.6–42.1%) (Table 2). The median PFS was 4.1 months (Fig. 1). The median OS time was 10.1 months (95% CI, 7.3–12.6 months), and the 1-year survival rate was 38% (Fig. 2).

Because the lower boundary of the 95% CI of the RR (15.6%) did not exceed the threshold RR (20%), the

Table 1 Patient characteristics ($n = 45$)

Age (years)	
Median	64
Range	36–75
Gender	
Male	33
Female	12
ECOG performance status	
0	24
1	21
2	0
Borrmann macroscopic type of primary cancer	
0	1
1	1
2	17
3	18
4	5
Unknown	3
Histological type	
Intestinal	25
Diffuse	18
Unclassified	2
Prior chemotherapy	
5-FU alone	18
S-1 alone	15
S-1 + CDDP	6
MTX + 5-FU	2
Others	4

ECOG Eastern Cooperative Oncology Group, *5-FU* 5-fluorouracil, *CDDP* cisplatin, *MTX* methotrexate

Table 2 Evaluation of response ($n = 45$)

Tumor response	Patients	
	n	% (95% CI)
Complete response	0	0
Partial response	13	28.9 (15.6–42.1)
Stable disease	17	37.7 (23.6–51.9)
Progressive disease	14	31.1 (17.6–44.6)
Not evaluated	1	4.4 (0–6.5)
Survival	Months (95% CI)	
PFS	4.1 M (2.5–5.7)	
OS	10.1 M (7.3–12.6)	

CI confidence interval, *PFS* progression-free survival, *OS* overall survival

MMC/CPT-11 combination as second-line chemotherapy could not be definitively concluded as efficacious for further investigation.

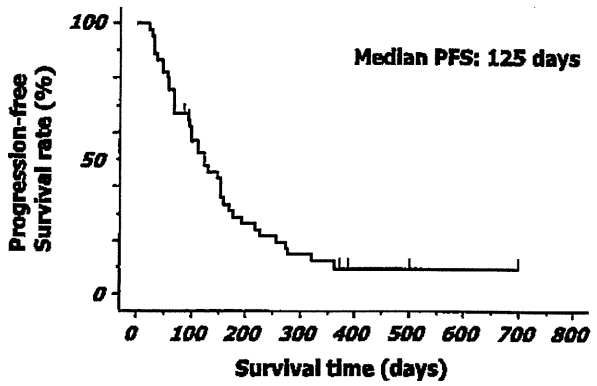

Fig. 1 Kaplan–Meier estimates of progression-free survival (*PFS*) rates

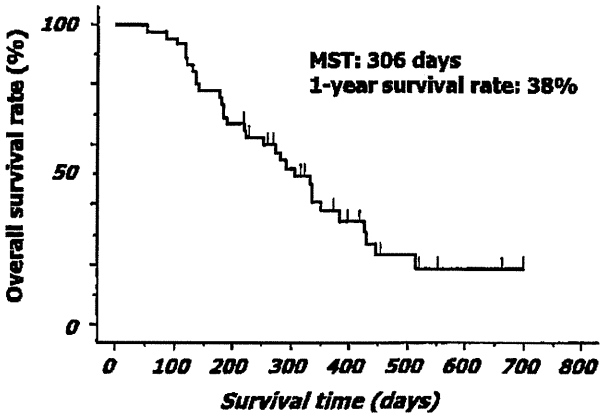

Fig. 2 Kaplan–Meier estimates of overall survival. *MST* Median survival time

Toxicity

The toxicities of the MMC/CPT-11 therapy are summarized in Table 3, with myelosuppression and gastrointestinal toxicity as major toxicities. Grade 3 and 4 neutropenia occurred in 24 and 29% of the patients, respectively, whereas grade 3 and 4 thrombocytopenia developed in only 7%. As for the nonhematological toxicities, the incidence rate of grade 3 diarrhea was 2%, and nausea and vomiting were mild. Early death due to interstitial pneumonitis within 30 days from the last chemotherapy occurred in 1 patient, which was considered by the JCOG Data and Safety Monitoring Committee to have been possibly related to the treatment.

Discussion

In second-line chemotherapy for AGC, the potential benefits remain unclear because of the few prospective studies that have been conducted thus far. These trials demonstrated that

Table 3 Grade 2–4 adverse events according to NCI-CTC ver. 2.0 ($n = 45$)

	Grade 2	Grade 3	Grade 4	Grade 3–4 (%)
Hematological WBC	24	8	5	29
Neutrophils	10	11	13	53
Hb	25	3	3	13
Platelets	1	2	1	7
Febrile neutropenia	0	4	0	9
Non-hematological Anorexia	13	11	0	24
Nausea	11	6	0	13
Diarrhea	4	1	0	2
Infection with grade 3/4 neutropenia	0	2	0	4
Infection without neutropenia	4	2	0	4

NCI-CTC National Cancer Institute-Common Toxicity Criteria, *Hb* hemoglobin

the RRs to second-line chemotherapy in phase II trials for gastric cancer were similar to those observed for other cancers which are more commonly treated after the failure of first-line chemotherapy. Furthermore, 2 Japanese randomized trials (i.e., SPIRITS [5] and JCOG9912 [6]) achieved a median OS of 13.0 months despite the relatively short median PFS of about 4–6 months. Although both JCOG9912 and our previous phase III study (JCOG9205 [19]) utilized 5-FU continuous infusion (c.i.) and 5-FU/CDDP, the obtained median PFS was 2 months and the OS in JCOG9912 was much longer than that in JCOG9205. In the present study, the proportion of patients who received second-line chemotherapy was >70%, which is higher than that obtained in our previous study (53%). The results of previous phase II trials consistently suggest that patients treated with second-line chemotherapy may survive longer than those provided with BSC, although the survival benefit of the second-line chemotherapy has not yet been clarified.

According to the 26 prospective phase II studies reported in the literature, obtained using the search expressions "gastric cancer" and "second-line chemotherapy" in PubMed, the average and median RRs were 18.8 and 20.0% (0–34.6%), respectively [18, 20–44]. Although the present study did not disprove the null hypothesis about RR, it is suggested that MMC/CPT-11 therapy with an RR of 28.9% may possess some antitumor activity as second-line chemotherapy.

As for survival, the present study showed a median survival time of 10.1 months (95% CI, 7.3–12.9 months), and a 1-year survival proportion of 38%. These data are similar to those obtained in the first-line chemotherapy setting and appeared to be better than those obtained using several other regimens, showing a survival period of 3.5–13 months compared with the reported median survival period of 7–10 months in untreated patients. However, it is very difficult to compare phase II studies due to differences in patient background and subsequent therapy. One reason for improved survival may be good clinical selection of a patient. At the baseline evaluation, the median age of the patients in the present study was 64 years (range, 36–75), and all the patients had a good PS of 0 or 1. Another reason for the improved survival was the high proportion of tumor stabilization (66.7%) after the administration of the MMC/CPT-11 regimen. Therefore, it is considered that MMC/CPT-11 therapy may provide some survival benefit.

The toxicity of the MMC/CPT-11 regimen can be considered tolerable and manageable. Hematological toxicity was within the expected range, including grade 4 neutropenia, observed in 13 patients (29%) and grade 3 febrile neutropenia in 4 patients (9%). According to a Japanese prospective pharmacogenomic study of CPT-11, homozygotes and double heterozygotes of *6 and *28 (*6/*6, *28/*28 and *6/*28) were significantly associated with severe neutropenia. The UGT1A1 gene test prior to receiving this regimen may be useful to decide the starting dose of CPT-11 or to decide whether the patient should receive CPT-11 and MMC combination chemotherapy or CPT-11 monotherapy [45]. Although treatment-related death was observed in 1 patient (2%) in the present study, the occurrence of adverse events was similar to that in JCOG9911-DI, a phase II study of the same regimen for colon cancer; thus, MMC/CPT-11 therapy was considered tolerable. In the present study, the proportion of patients with toxicity was similar to that of patients where MMC/CPT-11 therapy was used as second-line treatment against colorectal cancer [16].

From the above results, the present phase II study of MMC/CPT-11 therapy for FU-based chemotherapy-refractory gastric cancer is judged to be negative on the basis of the decision rule defined in the protocol. This may be due to the threshold RR being set very high owing to the lack of data as the basis for setting the threshold level and expected RR, because of the small number of phase II studies of second-line treatment when this protocol was developed. In fact, the RR cannot be considered poor compared with that in phase II studies performed in other treated patients (as shown in Table 2), with a favorable

survival time of 10 months. In conclusion, the MMC/CPT-11 regimen might be one treatment option for pretreated AGC in patients with good PS.

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Conflict of interest None.

Appendix

Investigators in participating institutions: Yamagata Prefectural Central Hospital, H. Saito; Tochigi Cancer Center, H. Fuji; Saitama Cancer Center, K. Yamaguchi; National Cancer Center Hospital East, T. Doi; Chiba Cancer Center Hospital, T. Denda; National Cancer Center Hospital Tokyo, Y. Shimada; Kitasato University East Hospital, W. Koizumi; Aichi Cancer Center Hospital, Y. Inaba; Nagoya Medical Center, H. Iwase; Osaka Medical College, H. Takiuchi; National Hospital Organization Shikoku Cancer Center, J. Nasu; Kumamoto Regional Medical Center Hospital, M. Yoshida.

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Phase II trial of preoperative S-1 plus cisplatin followed by surgery for initially unresectable locally advanced gastric cancer

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Abstract

Background: The aim of this study was to evaluate the efficacy and feasibility of preoperative chemotherapy with S-1 plus cisplatin in patients with initially unresectable locally advanced gastric cancer.
Methods: We enrolled patients with initially unresectable locally advanced gastric cancer because of severe lymph node metastases or invasion of adjacent structures. Preoperative chemotherapy consisted of S-1 at 80 mg/m^2 divided in two daily doses for 21 days and cisplatin at 60 mg/m^2 intravenously on day 8, repeated every 35 days. If a tumor decreased in size, patients received 1 or 2 more courses. Surgery involved radical resection with D2 lymphadenectomy.
Results: Between December 2000 and December 2007, 27 patients were enrolled on the study. No CR was obtained, but PR was seen in 17 cases, and the response rate was 63.0%. Thirteen patients (48.1%) had R0 resections. There were no treatment related deaths. The median overall survival time (MST) and the 3-year overall survival (OS) of all patients were 31.4 months and 31.0%, respectively. Among the 13 patients who underwent curative resection, the median disease-free survival (DFS) and the 3-year DFS were 17.4 months and 23.1%, respectively. The MST and the 3-year OS were 50.1 months and 53.8%, respectively. The most common site of initial recurrence after the R0 resection was the para-aortic lymph nodes.
Conclusions: Preoperative S-1 plus cisplatin can be safely delivered to patients undergoing radical gastrectomy. This regimen is promising as neoadjuvant chemotherapy for resectable gastric cancer. For initially unresectable locally advanced gastric cancer, new trials using more effective regimens along with extended lymph node dissection are necessary.

Keywords: Neoadjuvant chemotherapy; Lymph node dissection; Bulky lymph node; TS-1; Cisplatin; Para-aortic lymph node

Introduction

Gastric cancer is still one of the most common cancers in the world; 876,000 new cases were anticipated worldwide in the year 2000.[1] In Japan, 110,323 new cases were anticipated in the year 2003 and the 5-year survival rate of gastric cancer diagnosed from 1993 to 1996 was 54.4%.[2,3]

Currently, surgery remains the mainstay of curative treatment. However, only an R0 resection is associated with significant cure rates. Patients having microscopic (R1) or macroscopic (R2) residual tumor have an extremely poor prognosis.[4]

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Preoperative and neoadjuvant chemotherapy represent investigational options. The rationale of preoperative chemotherapy is based on the difficulty of performing an R0 resection in patients with initially unresectable locally advanced tumors and the high risk of micrometastatic disease in these patients. Neoadjuvant chemotherapy has potential for resectable gastric cancer for the purpose of treating micrometastases.

Intensive chemotherapy is necessary for the improvement of the R0 resection rate and complete elimination of the micrometastases. However, it is difficult for patients who undergo gastrectomy to tolerate intensive chemotherapy. Because weight decreases by gastrectomy, it is necessary to reduce the dose of chemotherapy. The tolerance to chemotherapeutic agents with digestive organ toxicity was often reduced by gastrectomy-related gastrointestinal effects.

S-1 (TS-1, Taiho Pharmaceutical, Tokyo, Japan) is an orally active combination of tegafur (a prodrug that is converted by cells to fluorouracil), gimeracil (an inhibitor of dihydropyrimidine dehydrogenase, which degrades fluorouracil), and oteracil (which inhibits the phosphorylation of fluorouracil in the gastrointestinal tract, thereby reducing the gastrointestinal toxic effects of fluorouracil) at a molar ratio of 1:0.4:1. The response rate of S-1 alone exceeded 40% in two phase 2 trials involving patients with metastatic gastric cancer.[5,6] The combination chemotherapy of S-1 plus cisplatin (CDDP) achieved a high response rate (74%, 95%CI: 54.9–90.6) in a previous phase I/II study of patients with metastatic gastric cancer.[7]

These factors led us to perform the current phase II trial to investigate the use of an active preoperative chemotherapy regimen. The primary objectives of the trial were to investigate tolerance to the preoperative regimen, its effects on operative morbidity and mortality, and the response rate. Secondary objectives included evaluation of the R0 resection rate, disease-free and overall survival, and failure pattern.

Patients and methods

Patients

The study was conducted as a prospective multi-institutional phase II trial by the Osaka Gastrointestinal Cancer Chemotherapy Study Group (OGSG) in Japan. All patients had histologically confirmed adenocarcinoma of the stomach. They also had to have initially unresectable locally advanced tumors because of invasion to adjacent structures or severe lymph node metastases, staged by contrast-enhanced CT as T2-3N2-3M0 or T4NanyM0, according to the Japanese Classification of Gastric Carcinoma (2nd English Edition).[8] They also had to have lymph node metastases that were measurable according to the RECIST[1.0] guidelines.[9] We did not require laparoscopic staging as an entry criterion for this study. Any sites of suspected M1 disease had to be ruled out prior to entrance into the study. No prior chemotherapy or radiation was allowed. The age range was 20–75 years. The performance status (ECOG) was 0 from 1.

Because of the worse prognosis of type IV gastric cancer, also known as scirrhous or linitis plastica, we excluded such cases.[10] Acceptable hematologic profile (WBC $\geqq$ 4000 cells/mm^3, hemoglobin $\geqq$ 8.0 g/dl, platelets $\geqq$ 100,000 cells/mm^3), and renal (BUN $\leqq$ 25 mg/dl, creatinine $\leqq$ 1.2 mg/dl and/or creatinine clearance > 60 ml/min) and hepatic function (total serum bilirubin < 1.5 mg/dl) were required. In addition, certain respiratory function test results (ratio of the forced expiratory volume in one second $\geqq$ 50%, PaO2 in room air $\geqq$ 70 mmHg) were required criteria. No clinically significant auditory impairment was allowed. Patients with prior cancer diagnosed during the previous 5-year period (except for colon carcinoma *in situ*) were excluded. Other exclusion criteria included significant cardiac disease, pregnancy or serious infections. The protocol was reviewed and approved by the Institutional Review Board of each institution. All patients gave written informed consent.

Preoperative chemotherapy

Patients found to have locally advanced gastric cancer as defined above, received two cycles of S-1 plus cisplatin every 35 days. Preoperative chemotherapy consisted of S-1 at 80 mg/m^2 divided in two daily doses for 21 days and cisplatin at 60 mg/m^2 intravenously on day 8. Physical examination, abdominal CT scan and assessment of toxicity were performed prior to each cycle. The response measurement of the preoperative chemotherapy was carried out according to the RECIST[1.0] guidelines. Because it was preoperative chemotherapy, response was not confirmed at least 4 weeks apart. Toxicity was recorded and graded according to the National Cancer Institution Common Toxicity Criteria (NCI-CTC) version 2.0 scale. Operative complication was graded according to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE v4.0). If a tumor decreased in size, according to protocol criteria, we added 1 or 2 more courses. If curative resection was considered possible after planned chemotherapy, the patient had surgery. If curative resection was considered difficult, a further course of chemotherapy was added. The doses of both agents were attenuated for grade $\geqq$3 toxicities, using standard reduction criteria.

Surgery

The surgery was planned for 3–6 weeks from the day of last administration of chemotherapy. Surgery involved a radical resection, the extent of which (total or distal gastrectomy) depended on the site of the primary tumor, with a D2 lymphadenectomy. We performed D2 or more dissection in patients with metastasis to N3 lymph nodes before chemotherapy. Spleen preservation in total gastrectomy procedure was entrusted to the decision of each clinician.