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Review Article

Topoisomerase I Expression in Tumors as a Biological Marker for CPT-11 Chemosensitivity in Patients with Colorectal Cancer

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Abstract
Irinotecan (CPT-11) is used as a first- and second-line chemotherapy for advanced or recurrent colorectal cancer (CRC). However, only 20%–30% of patients show an objective response to CPT-11 and the drug has severe toxicities, such as delayed-onset diarrhea, neutropenia, nausea, and vomiting. It is important to select patients who will demonstrate sensitivity to CPT-11 treatment to avoid unnecessary drug toxicities and to introduce anticancer treatment benefits to CRC patients. DNA topoisomerase I (Topo I) is essential for vital cellular processes such as DNA replication, transcription, translation, recombination, and repair. This article reviews the possibility of assessing Topo I protein expression in tumors as a biological marker for CPT-11 treatment in CRC.

Key words Chemosensitivity · Colorectal cancer · DNA topoisomerase I · Immunohistochemistry

Introduction

Colorectal cancer (CRC) is one of the most common cancers worldwide, and has undergone a rapid increase in incidence in Japan.[1] Advances in screening programs, surgical techniques, adjuvant chemotherapy, and surveillance programs have improved the 5-year survival of patients with CRC.[2] Moreover, recent advances in chemotherapeutic drugs have prolonged the survival of patients with unresectable advanced or recurrent CRC. Combination chemotherapy with oxaliplatin plus 5-fluorouracil/leucovorin (FOLFOX) or irinotecan (CPT-11) plus 5-fluorouracil/leucovorin (FOLFIRI) has become the standard regimen for unresectable advanced or recurrent CRC, and high response rates have been reported.[3–6]

CPT-11 is one of the key drugs for CRC.[7,8] CTP-11, a semisynthetic derivative of camptothecin, is activated in vivo to form SN-38, which is a potent topoisomerase I inhibitor and is 300–20000 times more cytotoxic than CPT-11.[9] However, the efficacy of CPT-11 is strongly limited by the development of drug resistance. Although treatment of advanced CRC patients with CPT-11 as a single agent has shown response rates of approximately 30%, these rates can reach 50% when used in combination with other agents.[10,11] Moreover, this drug has severe toxicities, such as diarrhea, nausea, vomiting, and neutropenia. Therefore it is necessary to identify new molecular markers that can identify the subset of patients who are unlikely to respond in order to improve the response rate to CPT-11 and to avoid the harmful toxicities of CPT-11 chemotherapy for patients with CPT-11-resistant tumors.

DNA topoisomerase I (Topo I) belongs to the DNA topoisomerase multimember family, which is essential for DNA topology modulation. Topo I transiently cleaves one strand of DNA, thereby allowing relaxation of the supercoiled DNA. This process is important during cell replication, translation, recombination, and repair.[12] Western and Northern blotting analyses have shown the Topo I protein and mRNA levels to be more abundant in several human tumors than in normal tissue.[13–16] Topo I is also a target for anticancer drugs. Topo I-inhibiting drugs, such as camptothecin and its derivatives,[17] interfere with the function of Topo I by binding to its active site and preventing religation of the DNA strand.[18] Camptothecin inhibits Topo I by forming stable Topo I–DNA cleavage complexes, and is specifically cytotoxic toward cells in the S phase.[19] In vitro, tumor cells with high Topo I protein levels respond better to Topo I inhibitors.[20] Moreover, a decreased Topo I content in cells is a frequent cause of resistance to camptothecin derivatives.[21,22]

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This article evaluates whether Topo I protein expression in primary tumors can act as a biomarker for chemosensitivity to CPT-11 in patients with recurrent CRC by reviewing several earlier studies and discussing previous data.

Topo I Protein Expression in Tissue

The clinical significance of Topo I protein expression has been intensively investigated by immunostaining formalin-fixed and paraffin-embedded tissue. Topo I protein is mainly detected in the nuclei of cells. Normal tissue shows an increased Topo I expression in the germinal centers of the tonsils and in the mucosal lymphocytes of the colon, and Topo I positivity is also detected in the glandular epithelium of the colon.[23] Topo I-positive cells are detected in the basal cell layer of normal skin.[24] Bauman et al.[25] and Hafian et al.[26] reported that Topo I and Topo II proteins are detected in normal tissues containing proliferating cells, including normal skin. Consequently, there is a close correlation between Topo I protein expression and cell proliferation.

An increased Topo I protein expression is detected in from 30% to 100% of cancer cells in ovarian carcinomas,[23] testicular tumors,[27] renal cell carcinomas,[28] gastric carcinomas,[29] breast carcinomas,[30] and oral squamous cell carcinomas.[26] Topo I protein expression is not detected in normal colorectal mucosa, and is mainly located in the nuclei of cancer cells.[24] Furthermore, Topo I protein expression was detected in 45 of 104 (43.2%) patients with primary CRC.[24] Therefore, Topo I protein expression is detected in almost 50% of CRCs.[31,32]

Topo I Protein Expression and Clinicopathological Findings in CRC

Data both in vitro and in vivo demonstrate that malignant cells with high proliferative activity can have high Topo I protein expression levels. Therefore, high Topo I protein expression is thought to correlate with tumor progression and a poor patient survival in CRC. The incidence of detectable Topo I protein expression increases with tumor progression in human sarcomas.[33] Topo I protein is more frequently detected in Dukes' C CRC tumors than in Dukes' A and B tumors, and Topo I protein expression is correlated with poor patient survival.[24] However, analyses of the relationship between Topo I immunohistochemical findings and clinical and pathologic parameters (T and N stages and differentiation) in oral squamous cell carcinomas showed only differentiation to be correlated with the Topo I expression rate.[26] In addition, Staley et al.[34] reported no correlation between the Topo I expression and Dukes' stage in 29 patients with CRC. Recently, Boonsong et al.[31] reported that Topo I protein expression in 249 primary CRCs was not correlated with sex, Dukes' stage, differentiation grade, survival status, p53 status, or status of proliferating cell nuclear antigen. Therefore, the clinical significance of Topo I protein expression in tumors is still unknown. Furthermore, the prognostic importance of Topo I in CRC remains controversial.

Topo I Protein Expression in Tumors and Effectiveness of CPT-11 Chemotherapy

Only 20%–30% of patients with CRC show an objective response to CPT-11. Moreover, patients who do not respond experience the toxicities of the drug. Consequently, there is a great need for new molecular makers that are capable of identifying the subset of patients who are unlikely to respond to CPT-11. In vitro studies have shown that tumors with higher Topo I protein levels respond to Topo I inhibitors, but Topo I mRNA expression is not predictive of the antiproliferative effects of Topo I inhibitors.[35,36] The ATP-binding cassette transporters designated ABCG2 and carboxylesterases are correlated with tumor sensitivity to Topo I inhibitors.[37–40] In addition, many molecular markers are associated with the response to CPT-11.[41] However, these data were obtained from in vitro analyses, and the clinical effectiveness of these markers has not yet been established in CRC. Therefore, further experiments are required before such molecular markers can be used in clinical settings.

A previous study investigated 23 Dukes' C patients who died from CRC recurrence, and reported that 16 were treated with CPT-11 just after the detection of cancer recurrence. The treatment protocol of CPT-11-based chemotherapy for these patients was an oral dose of 300 mg/m^2 per day of 1-(2-tetrahydrofuryl)-5-fluorouracil/uracil (1:4; UFT; Taiho Pharmaceutical, Tokushima, Japan) administered on days 1–28, followed by a 1-week rest during each course (35 days). CPT-11 was administered intravenously over 90 min at 100 mg/m^2 on days 1 and 15. Twelve of these patients had Topo I-positive primary tumors and the remaining four patients had Topo I-negative tumors. The survival periods after starting CPT-11 chemotherapy ranged from 2 to 43 months. The 50% survival periods after starting CPT-11 chemotherapy were 12 months in the 12 patients with Topo I-positive primary tumors, and only 4 months in the four patients with Topo I-negative primary tumors (Fig. 1). Although the number of patients in this study was small, the difference was significant ($P = 0.041$).[24] These findings suggest that Topo I protein expression in primary tumors may thus be a good indicator for the response to CPT-11 chemotherapy.

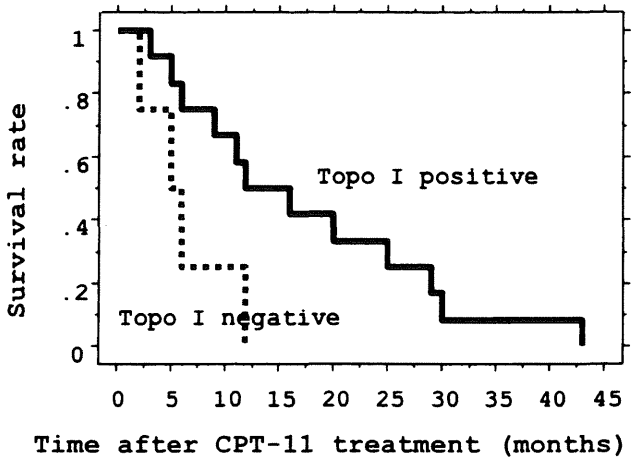

Fig. 1. Survival curves of 16 patients who were treated with irinotecan (*CPT-11*) just after the detection of colorectal cancer recurrence. The survival curve of the 12 patients with topoisomerase I (*Topo I*)-positive primary tumors (*solid line*) is significantly better than that of the four patients with Topo I-negative tumors (*dotted line*) ($P = 0.041$)

Topo I is a negative prognostic marker but at the same time a positive predictive marker in CPT-11 treatment in CRC. Topo I protein is detected mainly in proliferating cells and in tumors with high proliferative activity. The correlation between tumor Topo I expression and increased tumor progression was considered in CRC. Moreover, various tumors with high proliferative activity demonstrate high chemosensitivity.[42,43] Cells in S-, G2-, or M-phase of the cell cycle are more susceptible to applied chemotherapy. Therefore, the observation that Topo I is a negative prognostic marker is not contradicted by the fact that it is a positive predictive marker in CPT-11 treatment.

The Topo I protein levels in metastatic tumors from patients with CRC who were treated with 5-fluorouracil-based adjuvant chemotherapy are significantly increased in comparison with those in the primary tumors.[44,45] These findings may indicate that patients with recurrent CRC would benefit from Topo I targeting anticancer drug therapies. Dopeso et al.[46] reported that patients with absent or low levels of aprataxin in their tumors have better response rates and progression-free and overall survival rates than patients with moderate or high aprataxin levels among CRC patients treated with CPT-11. Aprataxin is a member of the histidine triad domain superfamily of nucleotide hydrolase and transferase, and participates in the repair of single- and double-stranded DNA breaks. Dopeso et al. emphasized that aprataxin will be a new molecular marker for a response to CPT-11 treatment. The sensitivity of tumors to Topo I inhibitors should therefore be extensively investigated to prolong the survival of patients with advanced or recurrent CRC and to prevent unnecessary harmful side effects of Topo I inhibitors.

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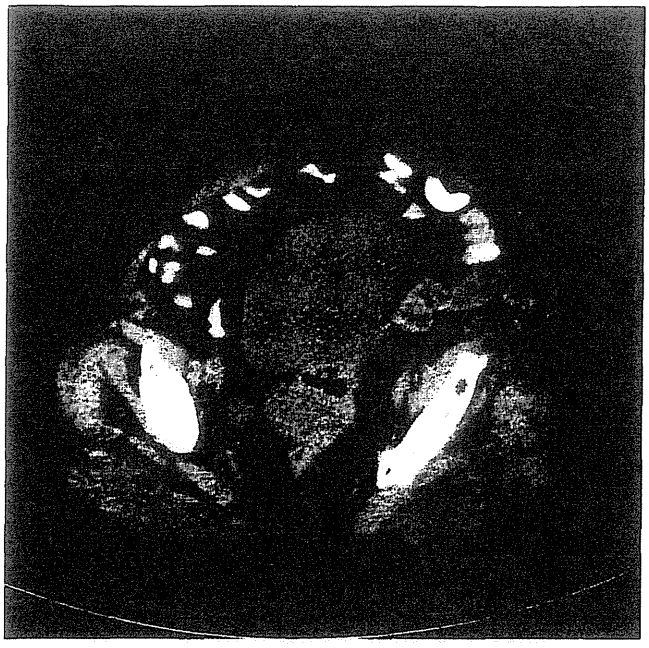

FIG. 1. An axial image of the mass with the abutting colon to the anatomic left.

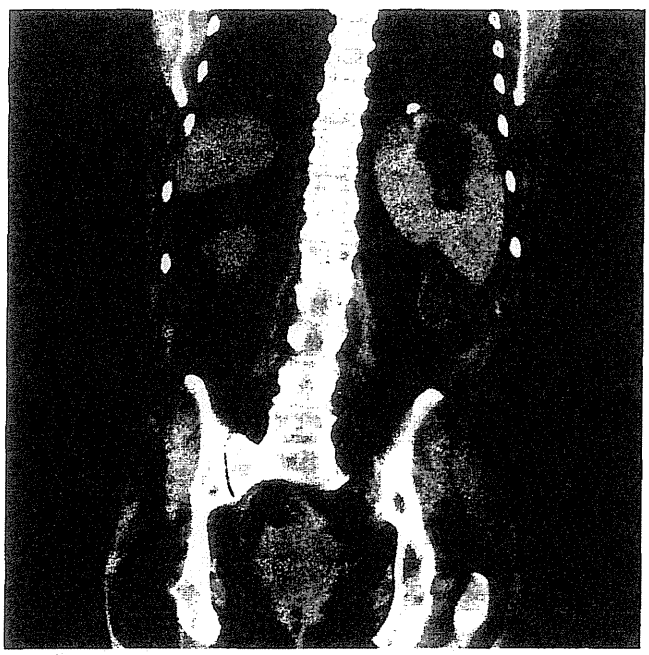

FIG. 2. A coronal image of the mass with similar relationship of the colon to the mass.

anterior mass was palpated on vaginal examination. Digital rectal examination was unremarkable. Colonoscopy revealed only a hyperplastic sigmoid polyp. A congenitally solitary kidney was hydronephrotic on computed tomography. An ostensible vaginal mass was suggested on cystoscopy. Radiologic biopsy was reported as adenocarcinoma consistent with colorectal origin. Computed tomography demonstrated that the pelvic mass was closely associated with the vagina but also involved the ureter and potentially the bladder (Figs. 1 and 2).

Inability to pass a ureteral stent because the mass obscured the ureteral orifice demanded a percutaneous nephrostomy. Given the involvement of the vagina, ureter, and bladder, a pelvic exenteration with urinary ileal conduit was performed. Grossly the tumor encased the ureter and invaded both the colon and vagina. Histologic examination revealed a poorly differentiated adenocarcinoma consistent with the prior biopsy. The postoperative course was uncomplicated.

This case of colonic adenocarcinoma arising in the vicinity of a previous pull-through procedure for an imperforate anus is rare. After surgical repair of this malformation, extraenteric mucosal rests are possible and unfortunately are not amenable to endoscopic surveillance. The patient's history remains paramount to guiding the investigation of related complaints. The exact operative details of this patient's original procedure are not known nor whether the anomaly was accompanied by a rectovestibular fistula.

In this case, the occult tumor presented at a late stage and created significant sequelae. Colonoscopy and digital rectal examination failed to explain the etiology of the patient's complaints. The only physical finding was a vaginal mass. This atypical presentation of colonic adenocarcinoma confirms the importance of understanding and thoroughly reviewing a patient's history.

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Outcome of Treatment of Liver Metastasis after Curative Surgery for Gastric Cancer

The efficacy of operative resection for liver metastases from colorectal cancer has been established. However,

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TABLE 1. *The Differences of Intervals Between Gastrectomy and Detection of Cancer Recurrence and Between Detection of Cancer Recurrence and the End of Follow-up According to the Types of Cancer Recurrence*

	No.	Average Interval Between Gastrectomy and Detection of Cancer Recurrence (months, mean ± SD)	Average Interval Between Detection of Cancer Recurrence and the End of Follow-up (months, mean ± SD)
Peritoneal	26	17.6 ± 13	7.6 ± 6.7[a]
Hepatic	18	20.4 ± 16.3	17.6 ± 14.8[b]
Lymph node	12	23.5 ± 19.4	13.5 ± 12.2[c]

a and b: $P = 0.004$; a and c: $P = 0.058$; b and c: $P = 0.437$.
SD, standard deviation.

the treatment of liver metastases from gastric cancer is controversial, because of the biologic aggressiveness of the disease. In addition, hepatic recurrence usually occurs in a combination of patterns, including peritoneal dissemination, lung metastases, and lymph node metastases. Only a few patients with liver metastases from gastric cancer are candidates for hepatic resection. Recent advancement of chemotherapeutic drugs has prolonged the survival of such patients with hepatic recurrence.[1] In this study, we retrospectively analyzed the outcomes of patients who developed liver metastases after curative surgery for gastric cancer to determine the outcomes of surgical intervention.

Between 1999 and 2008, 535 patients underwent curative (R0) gastrectomy for gastric adenocarcinoma at Tottori University Hospital. All patients were followed at our hospital until February of 2011. The type of cancer recurrence was determined by computed tomography and magnetic resonance imaging. Statistical analyses were performed using either the Mann-Whitney *U* test or the chi-squared test. All values with $P < 0.05$ were considered statistically significant.

A total 62 patients (11.6%, 48 male, 14 female; mean age, 68.7 years; range, 43 to 100 years) developed cancer recurrences. Peritoneal metastasis was found in 26 (4.9%), liver metastasis in 18 (3.4%), lymph node recurrence in 12 (2.2%), lung metastasis in three (0.6%), and bone metastasis in three (0.6%) patients. The mean interval between gastrectomy and detection of recurrence was similar for the different sites of cancer recurrence (peritoneal, hepatic, or lymph node recurrence). However, the prognosis of patients with hepatic or lymph node recurrence after detection of cancer recurrence was better than that of patients with peritoneal recurrence (Table 1).

Eighteen patients (14 male, 4 female; mean age, 70.7 years; range, 56 to 84 years) had recurrence in the liver. After gastrectomy, 10 patients (Stage II: 1 and III: 9) received adjuvant chemotherapy. Adjuvant chemotherapy did not prolong the time between gastrectomy and detection of hepatic metastasis (no adjuvant chemotherapy; n = 8; mean period, 28.4 months, adjuvant chemotherapy; n = 10; mean period, 19.4 months, $P = 0.328$). At the time of detection of liver metastasis, other types of metastases were found in four patients (peritoneal metastasis: 2 and lymph node metastasis: 2). After detection of liver metastasis, five patients refused additional treatment for cancer recurrence because of old age (n = 3) and poor performance status (n = 2). The remaining 13 patients were started on S-1-based chemotherapy.

Our criteria for hepatectomy in patients with hepatic recurrence from gastric cancer were: 1) age younger than 75 years; 2) good performance status (PS 0 to 1); 3) less than three metastatic tumors in the unilateral lobe; and 4) during S-1-based chemotherapy for 6 months, no new tumors appeared either inside or outside the liver. According to our criteria, three patients (mean age, 67 years) underwent hepatectomy after 6 months of S-1-based chemotherapy. One patient had three metastatic tumors in the right lobe and two patients had one metastatic tumor in the liver. The number and the size of metastatic liver tumors had not changed during S-1-based chemotherapy and the patients had no additional sites of cancer recurrence. Right hepatectomy was performed in the patient with three metastatic tumors, and partial hepatectomies were performed in two patients with a single metastatic tumor. Of the remaining 10 patients who did not have a hepatectomy, five were treated with only S-1 and 5 were treated with S-1 followed by another anticancer drugs. The mean survival time (MST) of five patients with best supportive care was 3.2 months and that of 10 patients treated with S-1-based chemotherapy was 18.7 months. However, no patient survived over 31 months. In contrast, of three patients who underwent hepatectomy after 6 months of S-1-based chemotherapy, two were alive 20 and 60 months after hepatectomy.

Recent advances in chemotherapeutic drugs have brought some hope for long-term survival of patients with hepatic metastases from gastric cancer. S-1 is a novel oral anticancer drug composed of tegafur (FT, a prodrug of 5-FU), gimestat (CDHP), and otastat potassium (Oxo) in a molar ratio of 1:0.4:1 and is based on the biochemical modulation of 5-FU. S-1 improves the tumor-selective toxicity of 5-FU by the modulatory actions of CDHP and Oxo.[2] A trial of S-1 plus cisplatin *versus* S-1 alone for first-line treatment of advanced gastric cancer (S-1 Plus cisplatin versus S-1 In RCT

In the Treatment for Stomach cancer [SPIRITS] trial) reported that the median overall survival was significantly longer in patients assigned to S-1 plus cisplatin (13 months) than in those assigned to S-1 alone (11 months).[3] From the result of the SPIRITS trial, S-1 plus cisplatin was recommended for the basic treatment of metastatic gastric cancer in Japan.

Our study indicated that S-1-based chemotherapy followed by hepatectomy prolonged survival time compared with S-1-based chemotherapy alone. Kakeji et al.[1] reported a hepatic resection rate of 17 to 38 per cent and the MST of patients was 12 to 34 months with a 5-year survival rate after hepatectomy of 18 to 42 per cent. A potentially curative hepatectomy may bring some hope of long-term survival for patients with hepatic metastasis. However, the reported survival rate after hepatectomy was rather unsatisfactory, because two thirds of the patients developed intrahepatic recurrence, and this high recurrence rate within 2 years of the surgery might suggest the presence of occult intrahepatic metastases even at the time of the hepatectomy. Thus, to avoid the possibility of intrahepatic recurrence or another type of recurrence after hepatectomy, we need to select the patients for hepatectomy. Thus, we started with S-1-based chemotherapy for patients with hepatic metastasis. During these chemotherapy periods (almost 6 months), patients who developed tumors in new regions or who showed enlargement of tumor size were excluded from hepatic resection.

We believe that surgical resection may bring some hope of long-term survival for selected patients with hepatic recurrence from gastric cancer.

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Indication of Emergency Operation and Intensive Care for Cardiopulmonary Arrest Related with Gastrointestinal Perforation

The survival rates of gastrointestinal perforation (GIP) have improved as the clinicopathological concept and practice guidelines for treating GIP, systemic inflammatory response syndrome, sepsis, and multiple organ dysfunction syndrome have improved as well as organ-supporting systems.[1] However, we often encounter patients with GIP who have already fallen into septic shock or cardiopulmonary arrest (CPA). Patients with severe peritonitis with unstable circulatory dynamics just before the CPA in itself are difficult to save. Although we often hesitate to perform surgery for these patients, this choice means withdrawal and withholding, basically.

We made a retrospective study of cases diagnosed with GIP from all CPA cases on arrival and just after arrival. We made a diagnosis of GIP based on findings in the operation and the autopsy, intestinal contents aspirated by abdominal paracentesis, and image findings of intraperitoneal free air with free fluid. Patients showing intraperitoneal free air without free fluid were excluded, whose intraperitoneal free air was often accompanied by pneumothorax and mediastinal emphysema. We used all information collected from arrival at the hospital to discharge. In patients with CPA just after arrival, we dealt with the duration from contact with the patients to arrival at the hospital as 0 minutes and substituted the initial rhythm with the rhythm at the CPA confirmation in the hospital. We examined the indication of resuscitation for CPA and the indication of surgery using the patients' medical records. Statistical analysis was performed using a *t* test and chi-squared test.

In our city, we established a unique system for the prehospital transfer of patients with CPA, who are transferred to the nearest of the selected 11 hospitals (12 since 2007), which received all patients with CPA regardless of the etiology, possibility for resuscitation, and any other reason except the predecision of transfer to a designated hospital. The data concerning CPA of these 11 hospitals can be population-based data, which considered a transportation network.[2, 3]

Of 12 subjects (four gastric, one duodenal, one small intestinal, four colorectal, and two unknown), four could not achieve return of spontaneous circulation (ROSC) (group unresuscitatable, group UR). The other eight patients could successfully achieve ROSC, four of whom were also able to undergo emergency surgery and be saved (operable group, group O), whereas the other four

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REVIEW ARTICLE

Second-line chemotherapy for gastric cancer: a new issue lies ahead in global trials

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Abstract Chemotherapy for gastric cancer has been advancing fairly well. It has been indicated that not only advances in first-line chemotherapy but also those in second-line chemotherapy have contributed to the prolongation of overall survival. The Arbeitsgemeinschaft Internistische Onkologie (AIO) study supports the idea that second-line chemotherapy is appropriate in patients with a good general condition. Also, the Japan Clinical Oncology Group (JCOG) integral analysis suggests that advances have been made in second-line chemotherapy. However, most recently reported studies of second-line chemotherapy have been conducted as small-scale phase II or retrospective trials. No randomized control trial to establish standard treatment has been reported. Which regimen is the most appropriate as second-line therapy must be investigated in the future. Currently, molecularly targeted agents for gastric cancer are being developed and tested in global trials. As a new issue in global trials, second-line chemotherapy has been emphasized. In recent global trials, subset analysis showed regional differences in overall survival. This was possibly associated with the regional differences in second-line chemotherapy. When developing new molecularly targeted agents for first-line chemotherapy, we cannot ignore the result that the proportion of patients in whom treatment was switched to second-line chemotherapy was high in Asia. In planning a global trial, this new issue should be sufficiently discussed.

Keywords Gastric cancer · Second-line chemotherapy · Global trial · Molecularly targeted agent

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Introduction

Gastric cancer is frequent in Asia, South America, and Eastern Europe, accounting for more than 800,000 new cases per year worldwide, and it is the second most common cause of cancer death [1]. Because early detection strategies are rarely practiced, except in Japan and Korea, most patients will present with advanced-stage disease, and will therefore need palliative chemotherapy. Some chemotherapy regimens have been established as first-line therapy, and some progress has been made in the treatment of advanced-stage disease [2–12]. However, almost all patients with metastatic gastric cancer will develop progressive disease (PD) after first-line therapy. With the availability of several active chemotherapy drugs, many patients who retain a good performance status after the initial treatment remain good candidates for additional therapy.

However, most clinical studies of second-line chemotherapy have been conducted as phase II, small-scale trials. The data obtained are limited, and there is no standard second-line chemotherapy. In this review, differing from previous reviews of second-line chemotherapy [13, 14], I have clarified the significance of second-line chemotherapy based on the recently reported results of randomized control trials of first-line chemotherapy. On the other hand, I refer to the concept of second-line chemotherapy as a potentially confounding factor in recent global trials.

Evidence for second-line chemotherapy

Chemotherapy for advanced/recurrent gastric cancer has been advancing fairly well. As evidence, the median survival in recent randomized comparative studies involving

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.

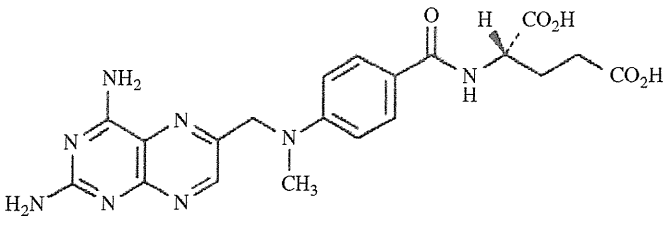

Fig. 1. Structure of methotrexate.

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

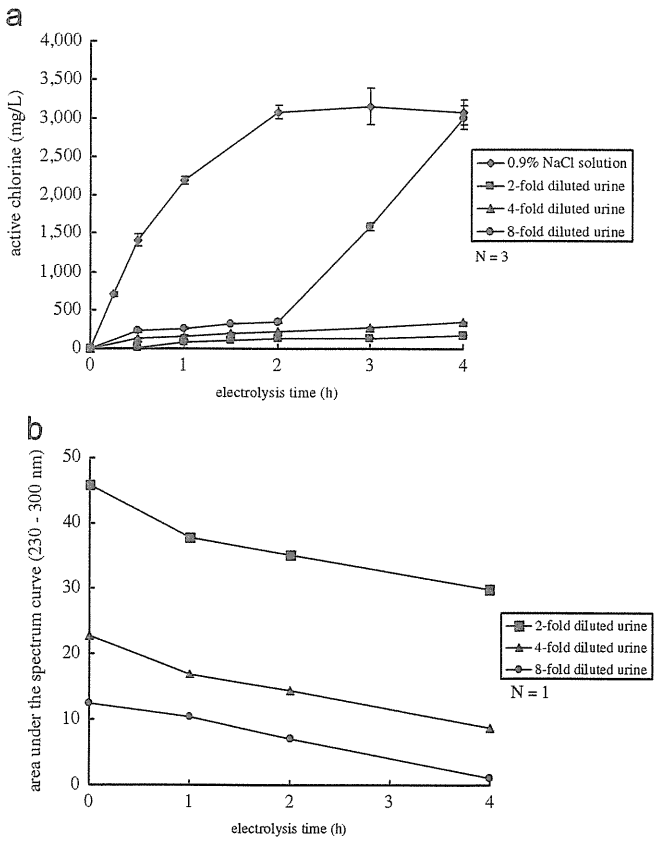

Fig. 2. Generation of active chlorine in the presence of urine (a) and the area under the spectrum curve between 230 and 300 nm of diluted urine (b) during electrolysis. Concentrations of active chlorine after electrolysis in the presence of one-half, one-fourth, and one-eighth part volumes of urine 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.

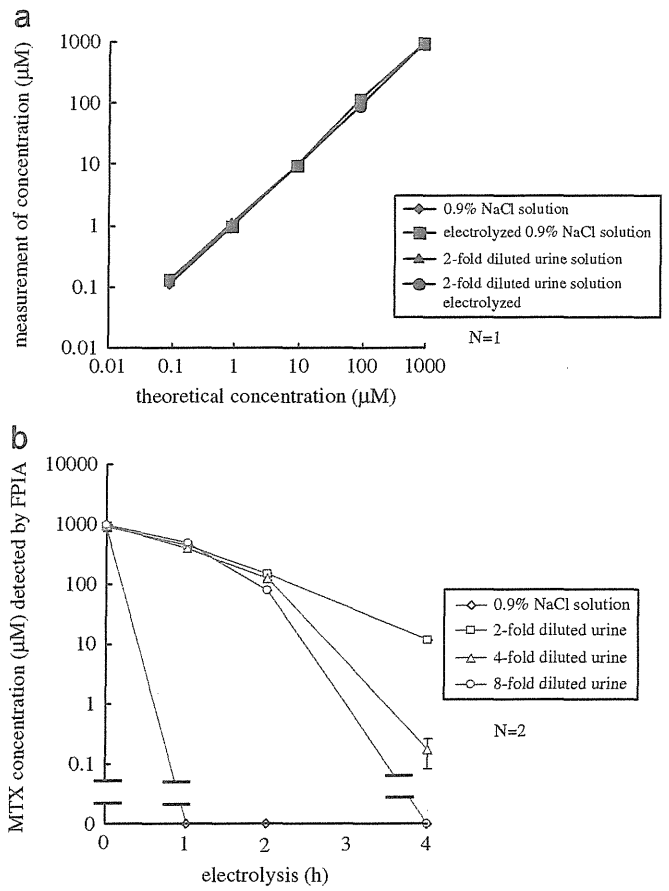

Fig. 3. Generation of calibration curves for MTX concentration determined by the FPIA method (a) and the destruction of MTX by electrolysis in the presence of urine (b). MTX (880.2 μM) was diluted with 0.9% NaCl solution, electrolyzed 0.9% NaCl solution, 2-fold-diluted urine, and 2-fold-diluted urine 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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