Table 3 Minor genetic variations detected in non-*UGT*+/+ patients who experienced grade 4 neutropenia

ID	Gene	Genetic variation	
		Nucleotide change (amino acid substitution)	Haplotype[a]
b1	*ABCB1*	304G>C (G102R)	*Block 1 *3*
b2(B)[b]		1804G>A (D602N)	*Block 2 *12*
b3(B)[b]		1342G>A (E448K)	*Block 2 *14*
b4		3043A>G (T1015A)	*Block 2 *16*
b5		3751G>A (V1251I)	*Block 3 *2*
c1	*ABCC2*	1177C>T (R393W)	**7*
g1	*ABCG2*	376C>T (Q126X)	*Block 1 *4*
g2		1465T>C (F489L)	*Block 2 *2*
g3		1723C>T (R575X)	*Block 2 *5*
s1(S)[c]	*SLCO1B1*	1007C>G (P336R)	
s2		311T>A (M104K)	
u1	*UGT1A1*	−3279T>G, 1941C>G	*#60-#IB* (+/+)

[a] Defined in previous papers for *ABCB1* [26], *ABCC2* [27], *ABCG2* [28] and *UGT1A1* [35]

[b] Linked with *ABCB1*2 (B)*

[c] Linked with *SLCO1B1*15 · 17 (S)*

occurred in *non-UGT*+/+ patients at rates of 8.0% (4/50) in the irinotecan monotherapy and 20% (11/55) in the cisplatin-combination therapy, we investigated possible contributions of these minor transporter variations and another low-activity *UGT*-haplotype, *UGT1A1#60-#IB* [35], to severe neutropenia.

Among the rare variations detected, eleven heterozygous transporter genetic variations and one *UGT1A1#60-#IB* homozygote were found in *non-UGT*+/+ patients who experienced grade 4 neutropenia (Table 3). These variations include an amino acid substitution leading to reduced in vitro activity, *ABCG2* 1465T>C (F489L) [36], and the stop codons, *ABCG2* 376C>T (Q126X) and 1723C>T (R575X) [28].

Additive effects of transporter gene haplotypes on neutropenia

Since multiple transporters are involved in irinotecan PK/PD, severity of toxicity might depend on the number and combinations of the low-activity variants, each of which does not effectively affect PD. To examine this possibility, we surveyed relationships between ANC nadirs and combinations of haplotypes associated with grade 3/4 neutropenia ($P < 0.1$) and the minor variations associated with grade 4 neutropenia (listed in the previous section); the data for selected haplotypes/variations are depicted in Fig. 2. For the combination therapy with cisplatin (Fig. 2b), homozygous *SLCO1B1*15 · 17* was included, but *ABCC2*1A* was excluded since its effect in the cisplatin-combination therapy was not consistent among the *UGT* genotypes.

In the irinotecan monotherapy, ANC nadirs in most patients with either one or more of *ABCG2#IIB*, *SLCO1B1*15 · 17* and the minor variations were lower than the median ANC nadirs of both *UGT*−/− and *UGT*+/− patients without them (None) (Fig. 2a). In particular, the effects were more evident in patients bearing two or more of the selected haplotypes/variations (including the *UGT*+). Among the patients who experienced grade 3 or 4 neutropenia, 80% of patients had two or more candidate haplotypes/variations in the *UGT* (−/− and +/−) group (Fig. 2a).

In *UGT*+/− patients with the cisplatin-combination therapy, ANC nadirs of the patients with *ABCB1*2/*2*, *ABCG2#IIB/#IIB*, *SLCO1B1*15 · 17/*15 · 17* or any minor variations, and their combinations were lower than the median values of patients without these markers (None), except for one patient with *ABCB1*2/*2* and *SLCO1B1*15 · 17* (*B/B* + *S*/−) (Fig. 2b). Also, in *UGT*−/− and *UGT*+/− patients, the effects were more evident in the patients with two or more of the selected haplotypes/variations. Among the patients who experienced grade 4 neutropenia, 82% of patients had two or more candidate haplotypes/variations in the *UGT* (−/− and +/−) group (Fig. 2b).

It was noted that the additive effect of *g1* [*ABCG2* 376C>T (Q126X)] was not observed in the heterozygotes (*g1*/−), but was evident in the compound heterozygotes with another *ABCG2* genetic polymorphism, *#IIB*, (*G/g1*) (Fig. 2a, b).

Regarding the combined effects of the above transporter genotypes on SN-38 AUC values, higher levels were observed in patients with the candidate haplotypes/variations of two or more genes in the monotherapy, but this trend was not always evident in the cisplatin-combination therapy patients (data not shown).

Discussion

In this study, we showed possible additive effects of transporter and *UGT1A1* genotypes on irinotecan PK and PD. Since multiple transporters are involved in irinotecan PK, it is likely that a functional alteration of one of the responsible transporters can be compensated by other transporters; thus, changes in PK/PD parameters by transporter genotypes may not always be large. However, the overall elimination rate of irinotecan or its metabolites might be altered under the conditions of simultaneously reduced activities of multiple transporters, higher irinotecan doses, or reduced UGT activity.

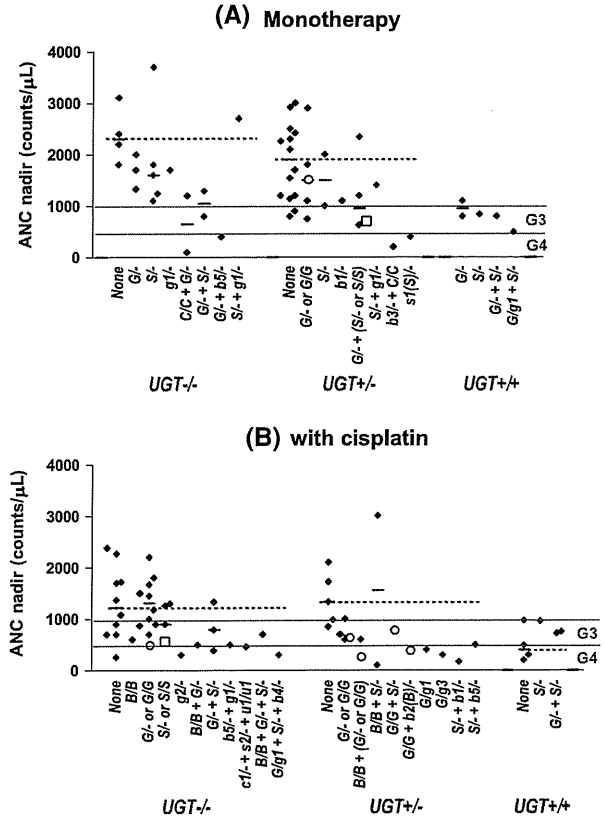

Fig. 2 Additive effects of transporter haplotypes/variations on ANC nadirs in irinotecan monotherapy (**a**) and combination therapy with cisplatin (**b**). *UGT+ = UGT1A1*6* or **28*; *B = ABCB1*2*; *C = ABCC2*1A*; *G = ABCG2#IIB* (*open circle*, *#IIB/#IIB*); *S = SLCO1B1*15 · 17* (*open square*, **15 · 17/*15 · 17*); *b1–u1* = minor variations listed in Table 3. **a** *None = non-(C, G, S* or *minors)*, **b** *None = non-(B, G, S* or *minors)*. The *bar* in each genotype represents the median. The *dotted lines* in each *UGT* genotype show the median values of patients without any selected transporter polymorphisms/variations (*None*). The *lines* (G3 and G4) represent the border of grade 3 and 4 neutropenia

In the irinotecan monotherapy, the increasing effect of *ABCB1*2/*2* (block 2) on SN-38 AUC/dose was evident while contributions of *ABCB1 BJL* (block 1), *ABCB1*1b* (block 3), *ABCG2#IIB* and *SLCO1B1*15 · 17* were not significant in the multivariate analysis. For neutropenia, additive effects were suggested for *ABCC2*1A/*1A*, *ABCG2#IIB*, *SLCO1B1*15 · 17*, and possibly some minor genetic variations in addition to *UGT1A1*6* or **28* (Fig. 2a). The association of *ABCB1*2* (block 2) with grade 3 diarrhea was also observed.

In the combination therapy with cisplatin, an increase in the SN-38 AUC/dose by *ABCB1*2* and for a decrease by *ABCB1*1b* were observed, but the multivariate analysis did not show their significant contributions. Regarding neutropenia, additive effects of *ABCB1*2/*2*, *ABCG2#IIB/#IIB*, and possibly, *SLCO1B1*15 · 17/*15 · 17* and some minor variations were suggested (Fig. 2b).

Thus, in both regimens, the associations of *ABCB1*2* (block 2) with higher SN-38 AUC/dose levels and toxicities (diarrhea or neutropenia), and additive effects of *ABCG2#IIB* and *SLCO1B1*15 · 17* with *UGT1A1*6* or **28* on neutropenia were observed. The current study also suggests that combination genotypes with two or more genes could have a greater effect on neutrophil count reduction than a single gene, indicating a quantitative property of multiple genetic factors affecting phenotype. These findings could partly explain a large interindividual variation in irinotecan toxicities within each *UGT* genotype.

In this study, influences of the transporter genotypes on SN-38 AUC/dose did not always correlate to an influence on neutropenia as observed in the combination therapy with cisplatin and in the case of *ABCB1*2* (block 2) in the monotherapy. Although weak negative correlations were observed between the SN-38 AUC level and ANC nadir, the SN-38 AUC values of patients who exhibited grade 3/4 neutropenia (ANC nadir < 1,000 counts/μL) were fairly diverse, especially in the combination therapy with cisplatin (Fig. 3). It is likely that the extent of toxicities depends not only on systemic exposure levels of the active metabolite for which hepatic UGT activity is a large contributor, but also on the elimination from the target cells (neutrophil progenitor cells or enterocytes) where transporter function might be more critical.

Our previous study showed the association of *ABCB1* block 2 **2* [1236C>T, 2677G>T (A893S) and 3435C>T] with lower renal clearance of irinotecan and its metabolites [16]. The current data obtained in the irinotecan monotherapy also suggest higher AUC/dose for irinotecan, SN-38G, and SN-38 with *ABCB1*2/*2*. Since a high affinity of P-gp for irinotecan is known, lower elimination rate of irinotecan could also result in higher plasma levels of its metabolites. Other studies have also suggested associations of the haplotype 1236T–2677T (corresponding to our **2* group in this study) with a reduced excretion rate of P-gp substrates [37] and SN-38 [25], and associations of the haplotype 2677T–3435T (corresponding to our **2* group in this study) with paclitaxel-induced neutropenia [38].

For *ABCC2*, *ABCC2* −1774delG, a tagging SNP of **1A*, was reported to be associated with low promoter activity and cholestatic or mixed-type hepatitis [32]. Patients with *ABCC2*1A/*1A* together with *ABCB1*2/*2* or *ABCG2#IIB* showed higher values of SN-38 AUC (Fig. 1) and neutropenia in the monotherapy (Fig. 2a), but these trends were not evident in the *UGT−/−* patients treated with cisplatin-combination therapy (data not shown). Thus, the effects of *ABCC2* might be dependent on combinations with other genetic and non-genetic factors. Conflicting clinical outcomes of *ABCC2* 3972C>T, a marker of **1C/G*, were reported to cause higher AUC of irinotecan and its

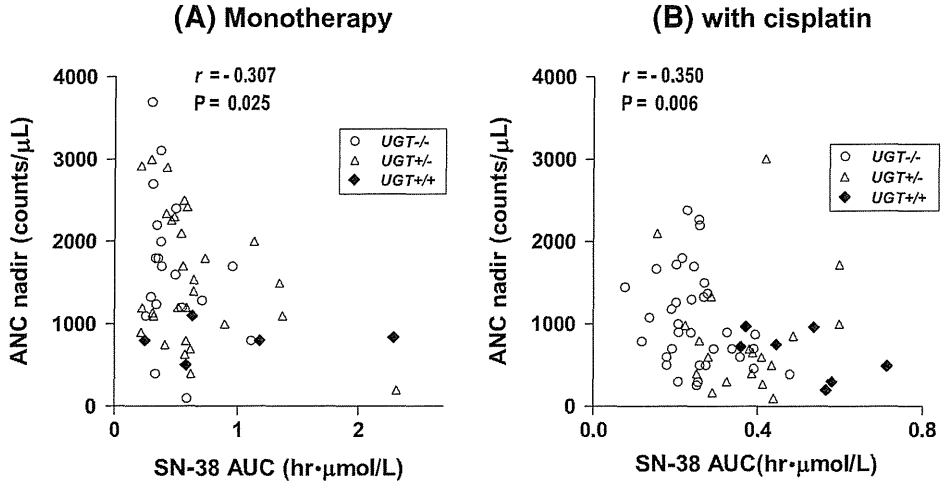

Fig. 3 Correlations between SN-38 AUC and ANC nadir in patients in irinotecan monotherapy (**a**) and combination therapy with cisplatin (**b**). *r* Spearman's rank correlation coefficient

metabolites in Caucasians treated with irinotecan monotherapy [18] and to lower the incidence of grade 3 diarrhea in Koreans treated with a combination therapy of irinotecan and cisplatin [24]. In the current study, no significant association of *ABCC2*1C/G* on PK/PD was observed in the monotherapy. Although a high incidence of grand 3/4 neutropenia was observed in patients with *ABCC2*1C/G* in the combination therapy with cisplatin, most patients also had *ABCG2#IIB* (data not shown); thus, the effect of *ABCC2*1C/G* remains obscure.

For *ABCG2*, the current study examined the association with the combinatorial haplotypes consisting of the three previously defined block haplotypes [28]. *ABCG2#IIB* contains the non-synonymous SNP 421C>A (Q141K), which was detected at higher frequencies in Asians and was reported to cause reduced expression of BCRP in vitro [36, 39–41]. In clinical studies, the association of 421C>A (Q141K) with higher plasma levels of diflomotecan was shown in Caucasians [42]. However, an association of this SNP with irinotecan PK/PD had not been shown [19, 24]. An association of 421C>A (Q141K) alone with irinotecan PK/PD was not significant in our hands (data not shown), but *#IIB* containing both 421C>A (Q141K) and IVS12 + 49G>T showed a moderate association with neutropenia. It is unclear whether the additional SNP IVS12 + 49G>T itself or another unknown linked SNP is causative for the reduced function. *ABCG2#IIIC* contains a non-synonymous SNP 34G>A (V12M) which has no influence on BCRP expression or activity in vitro [36, 39–41]. Our study showed no influence of *ABCG2#IIIC* on the SN-38 AUC/dose levels and neutropenia in the irinotecan monotherapy (data not shown), but did show a decreasing trend in grade 3/4 neutropenia in the combination therapy with cisplatin. In contrast, a report on Korean patients suggested the association of *ABCG2* 34G>A (V12M) with a higher incidence of grade 3 diarrhea in a combination therapy of irinotecan and cisplatin [24].

Among *SLCO1B1* polymorphisms, 521T>C (V174A), a tagging SNP of **15 · 17*, was demonstrated to reduce in vitro SN-38 influx [7], and clinical studies in Asians also showed its relevance to a higher SN-38 AUC and severe neutropenia in combination therapy of irinotecan with cisplatin [22–24]. Our results support these previous findings. Note that our **15 · 17* mainly consists of **17* [containing −11187G>A, 521T>C (V174A) and 388A>G (N130D)].

Taken together, the clinical data on transporter genotypes show variability among the studies. The reasons for these conflicting findings might be partly attributed to the ethnic differences in transporter genotypes and the regimens used. In addition, non-genetic factors, such as disease status and inflammation [43, 44], hepatic or renal function [45], and co-administered or pre-administered drugs, may also influence the clinical outcome.

The current study suggests combined effects of multiple haplotypes/variations on neutropenia. From clinical aspects of irinotecan therapy, the benefit of additional genotyping of transporters to predict severe toxicities should be clarified. Regarding grade 3 and 4 neutropenia, positive prediction values for two or more candidate genotypes including *UGT* (+) (Fig. 2) were 46 and 89% in the monotherapy and the cisplatin-combination therapy, respectively, which are low compared with *UGT+/+* (80 and 100%, respectively). Regarding grade 4 neutropenia, positive predictive values for these candidate genotypes were 15 and 41% in the monotherapy and the cisplatin-combination therapy, respectively, while for *UGT+/+*, they were 0 and 43%, respectively. Further studies using a

larger population size are needed to further elucidate the roles of these candidate markers.

In conclusion, the current study suggests there are additive effects for several transporter genotypes on the SN-38 AUC level and the reduction of neutrophil counts in irinotecan therapy. The clinical benefits of additional genotyping of these candidate markers should be further delineated.

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ORIGINAL PAPER

Sequential chemotherapy with methotrexate and 5-fluorouracil for chemotherapy-naive advanced gastric cancer with disseminated intravascular coagulation at initial diagnosis

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Abstract
Purpose Advanced gastric cancer (AGC) rarely presents with disseminated intravascular coagulation (DIC) at the time of diagnosis before treatment with no current standard chemotherapy (CTx) regimen. However the prognosis is extremely poor without CTx. We investigated the effectiveness of sequential CTx with methotrexate and 5-fluorouracil (MF) in chemotherapy-naive AGC patients with DIC.
Methods We retrospectively examined AGC patients who received first-line CTx and selected those who were diagnosed with DIC before starting CTx to investigate clinical characteristics and responses.
Results From July 1999 to January 2007, 1,365 patients with unresectable or recurrent AGC received first-line CTx at the National Cancer Center Hospital in Tokyo, Japan. DIC was diagnosed in 22 (1.6%) patients (16 men and 6 women; median age, 56 years) and the performance status of all the patients was 1/2/3 = 9/10/3. Nineteen patients (86%) had histologically diffuse-type adenocarcinoma and 18 (82%) had bone metastasis. Patients received sequential MF every week until progressive disease was confirmed, with DIC improving in 17 (77%) patients. The median time-to-treatment failure for AGC and overall survival were 98 days [95% confidence interval (CI), range 50–146 days] and 154 days (95% CI, range 126–180 days), respectively. Grade 3 or greater toxicities consisted of neutropenia (4 patients, 18%), anemia (9 patients, 40%), thrombocytopenia (4 patients, 18%), and bilirubinemia (1 patient, 5%).
Conclusions MF was an effective and well-tolerated regimen for improving DIC in chemotherapy-naive AGC patients with DIC; however, the prognosis of the patients remained poor even with improved DIC parameters.

Keywords Gastric cancer · Disseminated intravascular coagulation · Chemotherapy · Methotrexate · 5-fluorouracil

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Introduction

Disseminated intravascular coagulation (DIC) is a clinical condition in which various underlying diseases pathologically activate the coagulation system. DIC is characterized by multiple thrombi in microvessels (Levi and Ten Cate 1999; Sase et al. 2003). Subsequent microcirculation failure can induce organ injury, while exhaustion of coagulation factors and platelets induces a bleeding tendency. Underlying diseases causing DIC include hematological malignancies, infection, sepsis, and trauma. Solid tumors can be complicated by DIC (Al-Mondhiry 1975; Sallah et al. 2001), which occurs in approximately 10% of patients with solid tumors between the time of diagnosis and death (Okajima et al. 2000).

The prognosis of advanced gastric cancer (AGC) patients with DIC is extremely poor, and life expectancy without any intervention is only 1–3 weeks (Al-Mondhiry 1975; Sallah et al. 2001). DIC treatment includes chemotherapy (CTx) to control the underlying disease. However,

only a few studies have examined the effectiveness of CTx for AGC with DIC (Chao et al. 2000; Hironaka et al. 2000; Huang et al. 2008; Tokar et al. 2006).

One of the standard systemic chemotherapeutic regimens for unresectable or recurrent gastric cancer is 5-fluorouracil (5-FU) combined with cisplatin (CDDP) (Kim et al. 1993; Koizumi et al. 2008). However, when AGC is complicated by DIC, the patient's systemic condition is often poor with accompanying thrombocytopenia. Anemia is often detected and may be caused by microhemolysis (Jiang et al. 1997; Tsuchiya et al. 1989). An increased bleeding tendency due to thrombocytopenia is also often observed. These abnormal bleeding conditions make CDDP administration to AGC patients with DIC difficult.

The rationale for the use of methotrexate (MTX) in combination with 5-FU (MF) is based on biochemical modulation. Pre-administered MTX inhibits purine synthesis, which causes elevated levels of intracellular phosphoribosyl pyrophosphate that facilitate 5-FU metabolism, thereby enhancing its antitumor effects (Cadman et al. 1979; Fernandes and Bertino 1980). The effectiveness of MF against various cancers, particularly metastatic colon cancer, has been studied worldwide. A meta-analysis of 5-FU monotherapy and MF confirmed the efficacy of MF for colorectal cancer (Advanced Colorectal Cancer Meta-Analysis Project 1994) as well as for other unresectable and recurrent gastric cancers (Konishi et al. 1994; Perez et al. 1998).

MF is associated with only mild hematologic and nonhematologic toxicities and thus this regimen has been administered to patients in poor general condition, including those with AGC-induced ascites or peritoneal dissemination (Hamaguchi et al. 2008; Konishi et al. 1999; Tahara et al. 2001; Yamao et al. 2004). Based on some reports, in Japan, MF is considered one of effective and safety regimens for AGC patients in poor general condition. A randomized phase III study of 5-FU continuous infusion versus MF in chemotherapy-naive gastric cancer patients with peritoneal metastasis is currently being conducted by the Gastrointestinal Oncology Study Group of the Japan Clinical Oncology Group. In our hospital, we have been using MF as a first-line CTx for AGC patients with DIC.

In this study, we retrospectively investigated the therapeutic effects and toxicity of MF therapy in chemotherapy-naive AGC patients with DIC.

Patients and methods

Chemotherapy-naive AGC patients with DIC were identified among those receiving CTx for AGC at the National Cancer Center Hospital between July 1999 and January 2007. Chemotherapy-naive AGC patients included those with recurrent tumors for more than 6 months following completion of oral adjuvant fluoropyrimidine CTx. We analyzed patient background, treatment courses, response to MF therapy for DIC and AGC, time-to-tumor progression, and overall survival (OS). All study participants provided written consents before participating in the study.

Definition of DIC

We defined DIC according to the Japanese criteria issued in 1988 (Table 1) with individual patient scores based on underlying disease, bleeding symptoms, organ symptoms, and essential laboratory data, including elevated fibrin degradation product (FDP), decreased platelet count, decreased serum fibrinogen levels, and prolonged prothrombin times. DIC was diagnosed in patients with a total score of ≥ 7 points, and DIC was considered to improve when a patient's DIC score dropped to <5.

Chemotherapy regimen

MTX (100 mg/m^2) was administered intravenously by bolus infusion followed by a bolus infusion of 5-FU (600 mg/m^2) 3 h later. Six courses of leucovorin rescue (10 mg/m^2) were administered orally or intravenously every 6 h commencing 24 h following MTX administration.

Table 1 Diagnostic criteria of disseminated intravascular coagulation

Items/points	0	1	2	3
Basic disease	−	+		
Bleeding symptoms	−	+		
Organ symptoms	−	+		
FDP (mg/ml)	<10	≤10 to <20	≤20 to <40	≤40
Platelets ($\times 10^4$/mm^3)	<12	<8 to ≤12	<5 to ≤8	≤5
FIBG (mg/dl)	<150	<100 to ≤150	≤100	
PT (ratio)	<1.25	≤1.25 to <1.67	≤1.67	
Diagnosis	Total ≥7	Certain DIC		
	Total 6	Suspicion of DIC		
	Total ≤5	No DIC		

Disseminated Intravascular Coagulation Score of the Japanese Ministry of Health and Welfare in 1988

FDP fibrin degradation product, *FIBG* fibrinogen, *PT* prothrombin time

In an effort to prevent MTX-associated renal toxicities, acetazolamide (250 mg) was given intravenously immediately following MTX infusion, and sodium bicarbonate (33.3 mEq) was added to 500 ml of electrolyte solution and administered by drip infusion for urine alkalinization during the 3 h interval between MTX and 5-FU administrations. Treatment was repeated every week until progressive disease was observed in the patients.

Toxicity assessment

We evaluated each patient's physical examination records and laboratory tests at least every week during treatment, and a toxicity assessment was performed using the Common Terminology Criteria for Adverse Events version 3.0.

Evaluation of efficacy outcomes

Tumor response to CTx was assessed based on tumor reduction according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria for patients with measurable lesions. This objective response was evaluated every 4–8 weeks using a computerized axial tomography scan. The time-to-treatment failure (TTF) was measured from initiation to the final day of CTx, and OS was measured from CTx initiation to either the last date of follow-up or death and was censored as of the last date of contact. The TTF and OS were estimated using the Kaplan–Meier method, and statistical analyses were performed using Dr. SPSS II for Windows 11.0.1J software (SPSS Japan, Inc., Tokyo, Japan).

Results

Patients

A total of 1,365 chemotherapy-naive patients received CTx for recurrent or unresectable AGC from July 1999 to January 2007; 22 (1.6%) of these patients, including 16 men and 6 women, were diagnosed with DIC.

The background data of the 22 patients are summarized in Table 2. The median age was 56 years (range 26–75 years), the performance status (PS) was ≥2 in 13 of 22 cases (59%), and many patients had a poor clinical condition. In terms of histological type, diffuse-type adenocarcinomas were identified in 19 patients (86%) and macroscopic type 3 or type 4 tumors were seen in 16 patients (73%). Bone metastasis was found in 18 patients (82%).

MF was administered to all 22 patients. The median number of doses was eight (range 1–17) with 15 of the 22 patients (68%) receiving four or more doses of MF. Treatment was terminated because of disease progression in 21 patients, but one patient was still on MF at the time of this study.

Table 2 Patient characteristics

Characteristics	No. of patients	
	(n = 22)	(%)
Sex		
Male	16	73
Female	6	27
Age, years		
Median	56	–
Range	26–75	–
Histology		
Intestinal type	3	14
Diffuse type	19	86
Macroscopic type of primary tumor		
Early	3	14
Type 2	1	5
Type 3	8	36
Type 4	8	36
Unknown	2	9
ECOG performance status		
1	9	41
2	10	45
3	3	14
Gastrectomy		
No	10	45
Yes	12	55
Metastatic site		
Bone	18	82
Lymph node	11	50
Liver	3	13
Peritoneum	2	9

ECOG Eastern Cooperative Oncology Group

Safety and toxicity

Toxicities related to MF are summarized in Table 3. Hematological toxicities ≥grade 3 included neutropenia in four patients [(18%) although neutropenia-induced fever was not observed], anemia in nine patients (41%), and thrombocytopenia in four patients (18%). In terms of non-hematological toxicities ≥grade 3, one patient (5%) showed an elevated bilirubin level. However, none of the patients exhibited symptoms of gastrointestinal toxicities such as nausea, vomiting, or diarrhea.

Therapeutic outcomes

DIC improvement with a DIC score <5 was observed in 17 patients (77%). Following MF administration, most of the patients showed improvement of hematological data within

Table 3 Toxicities of MF regimen (no. of patients)

	Grade 1	Grade 2	Grade 3	Grade 4	Grade ≥ 3 (%)
Leukopenia	4	5	1	1	2 (9)
Neutropenia	4	4	3	1	4 (18)
Anemia	2	2	4	5	9 (41)
Thrombocytopenia	0	0	1	3	4 (18)
Bilirubinemia	1	1	1	0	1 (5)
Elevated AST/ALT	6	1	0	0	0
Elevated Creatinine	4	0	0	0	0
Nausea	10	1	0	0	0

2 weeks. Changes in platelet count, an important indicator in DIC assessment, are shown in Fig. 1. Tumor response based on the RECIST criteria could be assessed in only nine of these patients, and three of them (33%) showed evidence of a partial response. Median TTF was 98 days [95% confidence interval (CI), range 50–146 days] and median OS was 154 days (95% CI, range 126–180 days) (Fig. 2).

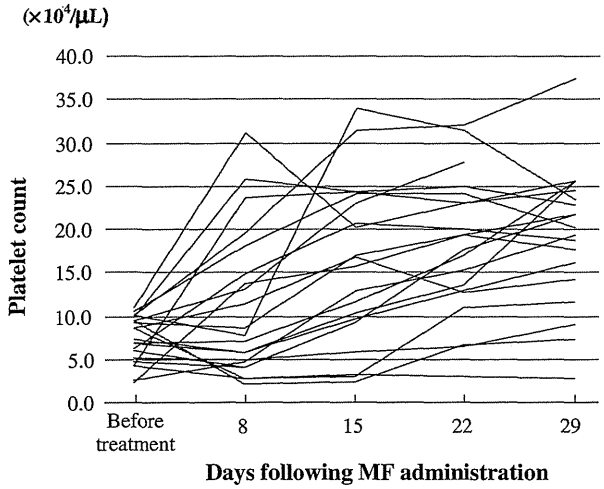

Fig. 1 Changes in platelet count during MTX + 5-FU

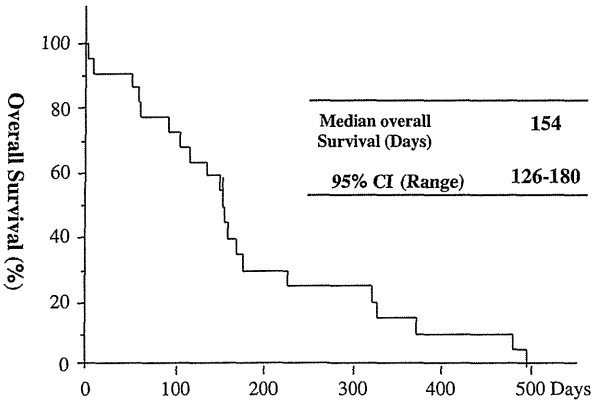

Fig. 2 Kaplan–Meier overall survival plot

Eleven of 17 patients (65%) whose DIC improved following MF administration later showed DIC recurrence at the time of disease progression. Despite MF administration, DIC showed no improvement in 5 of the 22 patients (23%). Weekly paclitaxel (PTX) was administered immediately as a second-line CTx to two of these five patients. Both responded to the PTX treatment, showing improvement in their DIC. Overall, CTx resulted in DIC improvement in 19 of 22 patients (86%). All patients who recovered from DIC were discharged and started outpatient chemotherapy. DIC showed no response to CTx in the remaining three patients (14%). One patient died of an acute subdural hematoma 2 days after starting MF, and another died of pulmonary carcinomatous lymphangiosis 8 days after starting MF. The third patient received MF four times. However, DIC showed no improvement, and the patient died from cancer progression 60 days after starting MF. We determined that these three patients were not treatment-related death but disease progression.

Discussion

To the best of our knowledge, this investigation is the largest single-institutional study of AGC complicated by DIC. Several other case series involving 6–19 patients (Chao et al. 2000; Hironaka et al. 2000; Huang et al. 2008; Tokar et al. 2006) are summarized in Table 4.

With respect to other solid tumors, DIC usually develops after diagnosis during the follow-up period; however, DIC can sometimes be detected during the initial diagnosis of metastatic gastric cancer (stage IV) or during recurrence after a curative surgical operation (Pasquini et al. 1995). In some patients, screening for DIC can lead to a diagnosis of AGC. There are several distinctive characteristics of AGC with DIC. In previous reports, relatively young patients were diagnosed with AGC with DIC. However, the patients in this study had a median age of 56 years. In terms of histological type, most of these patients had diffuse-type adenocarcinoma, ranging from 68 to 100%. Interestingly, there was a high frequency of bone metastasis or bone marrow involvement, ranging from 50 to 100%. In our study, bone

Table 4 Summary of case series previously reported

Author	Regimen	*n*	Median age (range)	Diffuse-type adenocarcinoma	Bone metastasis	DIC response	MST (weeks)
				No. of patients (%)	No. of patients (%)	No. of patients (%)	
Chao et al.	Weekly EEPFL	6	38 (36–71)	–	3 (50)	6 (100)	30
Hironaka et al.	MF	9	–	–	9 (100)	8 (89)	16
Tokar et al.	5-FU	6	48.5 (32–56)	6 (100)	–	5 (83)	14.5
Huang et al.	5-FU/Leucovorin	19	53 (31–72)	13 (68)	13 (68)	14 (74)	12
The present study	MF	22	56 (26–75)	19 (86)	18 (82)	17 (77)	22

DIC disseminated intravascular coagulation, *MST* median survival time, *EEPFL* etoposide and epirubicin and cisplatin and 5-FU, *MF* methotrexate and 5-fluorouracil

metastasis was diagnosed by bone scintigraphy and/or magnetic resonance imaging. Since such tests were performed only when symptoms were evident, the actual frequency of bone metastasis may have been higher. Although we did not conduct bone marrow tests, we suspect that most patients probably had bone marrow infiltration with the resultant bone marrow dysfunction leading to a predisposition to DIC development. In this case, AGC with DIC would be quite different from AGC without DIC.

When AGC is complicated by DIC, anti-cancer agents may not be used because of the poor general condition of the patient or the presence of thrombocytopenia and severe anemia. Unfortunately, the prognosis of untreated AGC with DIC is extremely poor, and patients generally live for only 1–3 weeks without CTx (Al-Mondhiry 1975; Okajima et al. 2000). Here, DIC improved in 19 of 22 patients following CTx with an OS of 167 days (95% CI, range 141–192 days), indicating that CTx was at least somewhat effective in treating AGC complicated by DIC. However, DIC showed no improvement in 3 of 22 patients, who died 2, 8, and 60 days following MF. Based on our analysis, we believe that MF provides a survival benefit for AGC patients with DIC. And 19 of 22 patients (86%) were discharged and continued outpatients chemotherapy, these data suggest that MF provide better QOL.

Several reports have been published examining the control of DIC by CTx in patients with AGC. Chao et al. administered etoposide, epirubicin, CDDP, and 5-FU to six AGC patients with DIC (Chao et al. 2000). Hironaka et al. (2000) administered MF to AGC patients with bone metastasis and reported DIC in nine of these patients. Tokar et al. reported that 5-FU administered alone and in combination with CDDP and epirubicin stopped the bleeding tendency in six AGC patients with DIC (Tokar et al. 2006). Finally, Huang et al. (2008) administered 5-FU and leucovorin to 19 AGC patients with DIC. Although each of these reports involved only a relatively small number of patients, the successful control of DIC by CTx was achieved in most patients. In our study, MF improved DIC in 17 of 22 patients (77%). Based on our results, AGC patients with DIC may respond favorably to CTx with accompanying improvement in DIC. However, even if DIC improves in these patients, their prognosis still appears to be worse than that of AGC patients without DIC.

One of the primary reasons for selecting MF is because of its mild toxicity (Hamaguchi et al. 2008; Konishi et al. 1999; Tahara et al. 2001; Yamao et al. 2004). However, the frequency of both anemia and thrombocytopenia as adverse events was higher in the present study patient group than in past clinical study patient groups. Anemia may have been caused by microhemolysis or the bleeding tendency associated with DIC. CTx improved thrombocytopenia (Fig. 1), and the other toxicities were mild and well-tolerated by the patients.

In conclusion, MF for the treatment of chemotherapy-naive AGC patients was an effective and well-tolerated regimen for improving DIC; however, the prognosis of the patients remained poor even with improvement in DIC parameters. Although this was a retrospective study where concrete conclusions based on our findings are not possible, the results are nonetheless significant in terms of their implications for clinical practice.

Conflict of interest statement We received no financial support for this study and report no conflicts of interest. Informed consent was obtained from all patients before initiating chemotherapy. This study was approved by the president of National Cancer Center Hospital.

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ORIGINAL ARTICLE

Effects of bevacizumab on plasma concentration of irinotecan and its metabolites in advanced colorectal cancer patients receiving FOLFIRI with bevacizumab as second-line chemotherapy

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Abstract
Purpose Bevacizumab (BV) prolongs the survival of colorectal cancer patients when combined with irinotecan (CPT-11)-based regimens. In the AVF2107g study, the area under the curve (AUC) ratio for bolus CPT-11/5-fluorouracil (5-FU)/leucovorin (LV) (IFL) with the BV arm to bolus IFL with placebo indicated that SN-38 concentrations may have been increased in subjects receiving BV. However, the mechanism underlying such increase remains unclear, and the difference might be caused by an imbalance between the two arms and a possible inter-subject variability of CPT-11 metabolism. Within-subject comparisons were used to evaluate the effect of BV on advanced colorectal cancer patients when administered with the FOLFIRI regimen as second-line chemotherapy.
Methods Ten advanced colorectal cancer patients received the FOLFIRI regimen every 2 weeks. At cycle 1, BV was administered following FOLFIRI administration to allow baseline pharmacokinetic (PK) analysis of CPT-11 and its metabolites. From cycle 2, BV was administered just before FOLFIRI administration. Plasma samples were collected under the same condition (at cycle 3).
Results There were no significant differences in the C_{max} and $AUC_{0-\infty}$ of CPT-11, SN-38, and SN-38G between cycle 1 (without BV) and cycle 3 (with BV). PK parameters of CPT-11, SN-38, and SN-38G were not significantly affected by BV. There were no significant differences in the changes in the AUC ratio of CPT-11 to SN-38 between cycles 1 and 3, as well as in the ratio of SN-38 to SN-38G.
Conclusion BV does not affect the plasma concentration of CPT-11 and its metabolites on FOLFIRI regimen.

Keywords Bevacizumab (BV) · Irinotecan · Pharmacokinetics · Colorectal cancer

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Introduction

Bevacizumab (BV) is a humanized monoclonal antibody against vascular endothelial growth factor, an important regulator of physiologic and pathologic angiogenesis [1]. A large, randomized, controlled Phase III clinical trial (AVF2107g) has demonstrated that BV addition to standard chemotherapy with the bolus irinotecan (CPT-11)/5-fluorouracil (5-FU)/leucovorin (LV) (IFL) regimen improves survival of patients with previously untreated metastatic colorectal cancer [2]. Subsequently, CPT-11/bolus 5-FU/continuous 5-FU/LV (FOLFIRI) + BV conferred a significant survival benefit compared with IFL + BV in the BICC-C study [3]. Thus, CPT-11 with BV demonstrated significant survival benefits in patients with colorectal cancer. CPT-11 has a complex metabolism requiring activation into SN-38 by carboxylesterase [4, 5] and glucuroconjugation for catabolism [6]. As shown in the AVF2107g study, SN-38 concentrations were on average 33% higher in patients receiving bolus IFL in combination with BV compared with bolus IFL alone [7]. However, the underlying mechanism of such increase remains unclear, and the difference might be caused by an imbalance between the two arms and a possible inter-subject variability of CPT-11 metabolism. Thus, we investigated the potential pharmacokinetic (PK) interaction between CPT-11 and BV in advanced

colorectal cancer patients when administered with the FOLFIRI + BV regimen as second-line chemotherapy.

Methods

Inclusion and exclusion criteria

Patients meeting the following inclusion criteria were eligible: histologically proved colorectal cancer (e.g., adenocarcinoma, mucinous carcinoma, and signet-ring cell carcinoma); failure of first-line treatment containing 5-FU-based chemotherapy (almost an adjuvant setting and recurrence were found in the chemotherapy period or after the end of chemotherapy within 24 weeks) or oxaliplatin-based chemotherapy (all FOLFOX regimens) without BV and CPT-11; Eastern Cooperative Oncology Group performance status of 0–2; age: 20–74-year-old; no previous exposure to BV or CPT-11; adequate bone marrow function (leukocyte count $\geq$3,000 and $\leq$12,000/μl, hemoglobin $\geq$8.0 g/dl, and platelet count $\geq 10 \times 10^4$/μl); serum creatinine level $\leq$1.5 mg/dl; total bilirubin level $\leq$1.5 mg/dl; AST and ALT $\leq$100 IU/l; qualitative urine protein $\leq$(1+); measurable disease according to response evaluation criteria for solid tumors (RECIST); and written informed consent.

Patients were excluded if they had the following: known central nervous system metastasis; other active double cancer; inadequately controlled hypertension, diarrhea, diabetes, or heart disease; severe peritoneal metastasis; interstitial pneumonia or pulmonary fibrosis; previous history of vascular thromboembolism or severe drug hypersensitivity; bleeding tendency; hepatic B or C virus infection; underwent any form of surgery within 4 weeks before study enrollment; pregnant or lactating.

Study design

Ten patients were treated with the FOLFIRI regimen preceded by BV every 2 weeks. At cycle 1, CPT-11 was administered before BV to allow baseline PK analysis of CPT-11 and its metabolites. At cycle 3, plasma samples were collected for PK analysis of CPT-11 when administered in combination with BV. The PK investigations were used intra-patients comparison.

Pretreatment and follow-up examination

Complete medical history evaluation, physical examination, laboratory tests (complete blood count, creatinine, serum electrolytes, calcium, uric acid, total protein, albumin level, hepatic, and coagulation tests) and urinalysis were performed to obtain baseline data and repeated biweekly.

Toxicity was evaluated biweekly and graded using the National Cancer Institute's Common Toxicity Criteria, version 3.0. Tumor responses were evaluated and measured as baseline data and reassessed every 4 cycles using RECIST.

Drug administration

The FOLFIRI regimen consisted of CPT-11 (180 mg/m^2 IV over 90 min), *l*-LV (200 mg/m^2 IV over 2 h), and 5-FU (400 mg/m^2 IV bolus), followed by 5-FU (2,400 mg/m^2 IV over a 46-h infusion), and repeated every 2 weeks. BV was administered as a 30-min intravenous infusion at a biweekly dose of 10 mg/m^2 before the FOLFIRI regimen (only in the cycle 1, BV was administered after the FOLFIRI regimen for PK analysis of the non-BV phase) (Fig. 1).

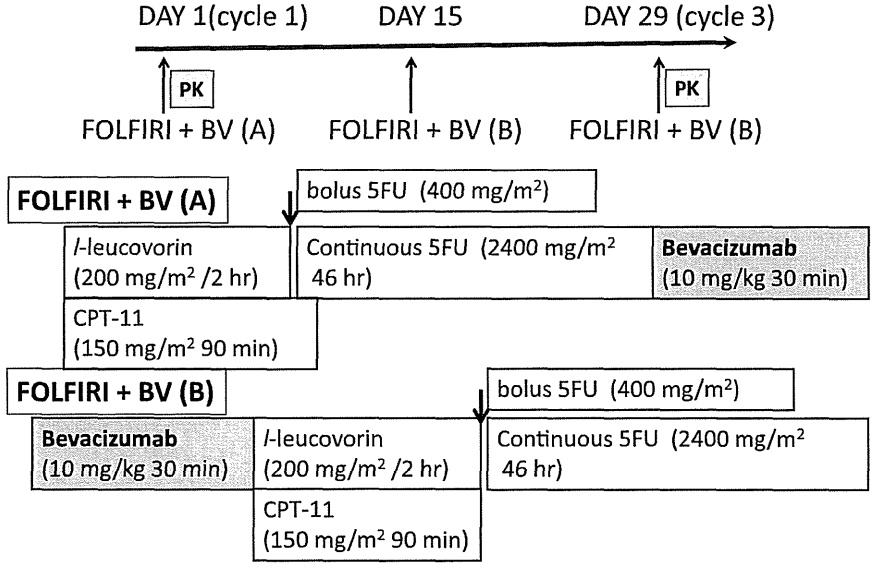

Fig. 1 At cycle 1, CPT-11 was administered before BV to allow baseline pharmacokinetic (PK) analysis of CPT-11 and its metabolites. At cycle 3, plasma samples were collected for PK analysis of CPT-11 when administered in combination with BV

Table 1 Patient characteristics

Age (years)	
Range	38–74
Median	60
Gender	
Male	9
Female	1
Previous chemotherapy	
5-FU-based regimen[a]	5
FOLFOX	5
Total cycles of treatment	
Range	7–19
Median	11

[a] As adjuvant chemotherapy

Pharmacokinetic analysis

Plasma samples were collected at cycles 1 and 3 before the start of chemotherapy, and 0, 1, 2, 4, 7, and 24 h after CPT-11 infusion. Whole blood (4.0 ml) samples were collected in heparinized tubes and centrifuged at 3,000 rpm for 10 min at 4°C. Then, 2.0 ml of plasma was transferred into tubes with 2.0 ml of phosphate buffer (0.1 M) and stored at −80°C before analysis. Thereafter, quantitative analysis of CPT-11 and its metabolites was performed using high-performance liquid chromatography [8]. The lower limit of quantification was 0.002 μg/ml for CPT-11 and its metabolites. Maximum plasma concentration (Cmax), area under the plasma/serum concentration time curve (AUC) and terminal half-life were determined. The AUC calculation is limited up to 24 h of to infinite (∞). Changes in the ratios of CPT-11 to SN-38 and SN-38 to SN-38G were estimated as $AUC_{SN\text{-}38}/AUC_{CPT\text{-}11}$ and $AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$, respectively.

Statistical analysis

Correlation between related species were all carried out using the paired *t* test (Microsoft Excel 2000 SP-3), and *P* values <0.05 with a two-tailed distribution were considered significant.

Results

Patient characteristics

Ten patients received the treatment regimens (Table 1), and all the patients completed the PK program and were assessable for drug safety and anti-tumor activity. A total of 120 cycles of treatment was administered (median number of cycles: 11 (range 7–19)).

Pharmacokinetic analysis

Analysis of the PK parameters showed no significant difference between the parameters of cycle 1 (non-BV phase) and cycle 3 (BV phase) (Table 2). This indicates that BV had no effect on the pharmacokinetics of CPT-11. The mean AUCs for CPT-11 were 12.2 ± 2.3 μg h/ml at cycle 1 and 12.8 ± 1.7 μg h/ml at cycle 3. The half-lives of CPT-11 were 6.0 ± 0.6 h at cycle 1 and 5.7 ± 0.6 h at cycle 3. Mean CPT-11 concentrations versus time profiles either alone or in combination with BV were nearly superimposed (Fig. 2).

The mean SN-38 PK parameters showed no significant differences between cycles 1 and 3 (Table 2). The mean AUCs for SN-38 were 0.40 ± 0.44 μg h/ml at cycle 1 and 0.22 ± 0.16 μg h/ml at cycle 3. Mean SN-38 concentrations versus time profiles either alone or in combination with BV were nearly superimposed (Fig. 3). In SN-38G, significant differences in the PK parameters were also not found between cycles 1 and 3 (Table 2), and mean SN-38G concentrations versus time profiles either alone or in

Table 2 Pharmacokinetic parameters

Analyte		C_{max} (mg/ml)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (mg h/ml)	$MRT_{0-\infty}$ (h)	Vd (L)	CL (L/h)
CPT-11	BV (−)	2.1 (0.3)	1.5 (0)	6.0 (0.6)	12.2 (2.3)	6.1 (0.6)	185 (43.3)	21.6 (5.6)
	BV (+)	2.1 (0.3)	1.5 (0)	5.7 (0.6)	12.8 (1.7)	6.1 (0.5)	164 (34.6)	19.7 (3.0)
SN-38	BV (−)	0.024 (0.013)	2.0 (0.7)	14.3 (16.6)	0.40 (0.44)	–	–	–
	BV (+)	0.022 (0.012)	2.8 (0.8)	8.3 (7.6)	0.22 (0.16)	–	–	–
SN-38G	BV (−)	0.14 (0.030)	2.4 (0.3)	12.9 (4.7)	1.98 (0.70)	–	–	–
	BV (+)	0.14 (0.030)	2.6 (0.6)	11.4 (3.5)	1.81 (0.26)	–	–	–

Values are expressed as mean (±SD). There are no significant differences in the C_{max} and $AUC_{0-\infty}$ of CPT-11, SN-38, and SN-38G between cycle 1 (BV-) and cycle 3 (BV+); paired *t* test

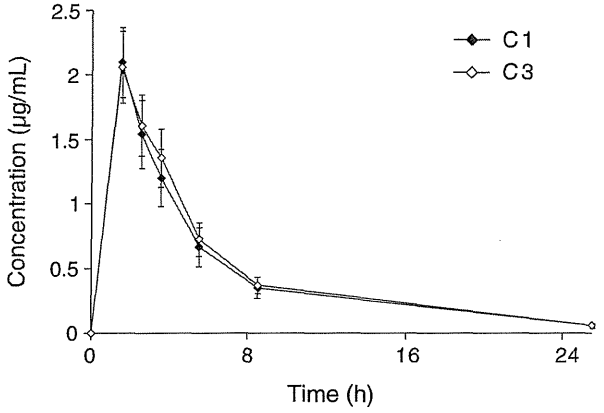

Fig. 2 Mean CPT-11 concentrations versus time profiles either alone or in combination with BV were superimposed

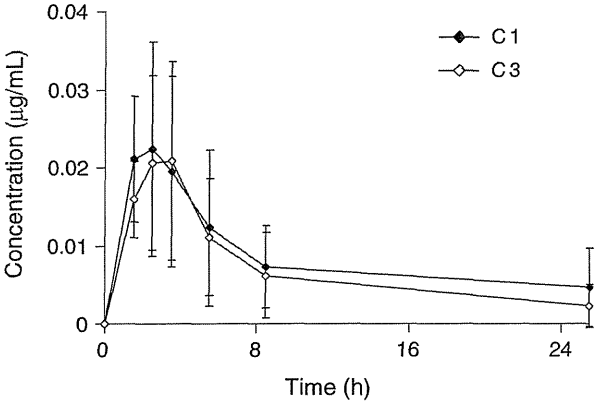

Fig. 3 Mean SN-38 concentrations versus time profiles either alone or in combination with BV were nearly superimposed

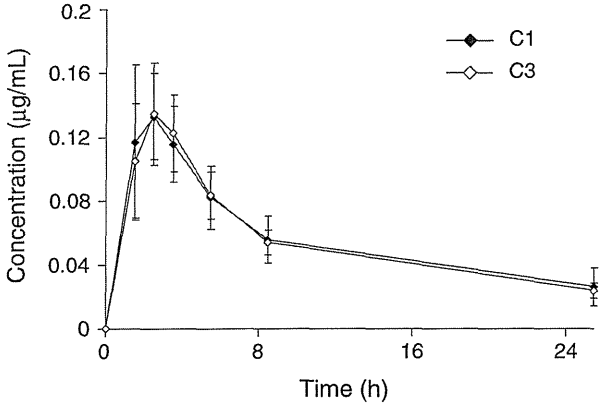

Fig. 4 Mean SN-38G concentrations versus time profiles either alone or in combination with BV were superimposed

combination with BV were also nearly superimposed (Fig. 4).

There were no significant differences in the changes in the ratio of CPT-11 to SN-38 between cycles 1 and 3 (Table 3), as well as in the ratio of SN-38 to SN-38G.

Table 3 Changes in ratio of CPT-11 to SN-38 and SN-38 to SN-38G

Patient No.	AUC ratio of SN-38/CPT-11(%)		AUC ratio of SN-38G/SN-38	
	BV (−)	BV (+)	BV (−)	BV (+)
1	3.1	4.1	3.8	3.9
2	2.2	2.2	7.9	5.9
3	2.5	1.8	8.7	8.0
4	9.2	1.8	2.3	7.7
5	0.6	0.7	23.1	22.3
6	0.3	0.3	51.7	76.0
7	4.4	1.4	3.5	8.8
8	1.0	0.5	13	23.8
9	1.0	0.8	13.5	14.6
10	5.6	4.3	2.3	3.2

There were no significant differences in the AUC ratios of SN-38/CPT-11 and SN-38G/SN-38 between cycle 1 (BV-) and cycle 3 (BV+); paired *t* test

The results indicate that the CPT-11 and BV combination had no effect on the extent of conversion of CPT-11 into its metabolites SN-38 and SN-38G.

We also observed a larger inter-patient variability for the changes in the ratios of CPT-11 to SN-38 and SN-38 to SN-38G (Table 3).

Discussion

In the present study, we found no significant differences in the mean AUCs, C_{max} and CPT-11 clearance after BV addition. Our results demonstrate that BV addition to CPT-11 (in the FOLFIRI regimen) showed no effect on the drug disposal of CPT-11 and its metabolites. This is the limited sample size study, but this is the first report clarifying the effect of BV on CPT-11 metabolism in humans.

Gaudreault et al. previously reported on the effect of BV on CPT-11 metabolism and safety using cynomolgus monkeys as subjects. Their report was the only published study available in the literature search regarding the effect of BV on CPT-11 metabolism. In their study, monkeys received bolus IFL with or without BV, and blood samples were collected for PK analysis of CPT-11 and 5-FU. They concluded that BV had no effect on the metabolism of either agent, although the number of animals tested in each group was small [with BV ($n = 5$); without BV ($n = 4$)] and no statistical comparison between groups was performed [9].

As previously shown, in the AVF2107g study, CPT-11 metabolism was characterized in a small PK study (results are presented only in the package insert of BV

[7]). In the results, SN-38 concentrations were on average 33% higher in patients receiving bolus IFL in combination with BV compared with bolus IFL alone. But it might be caused by an imbalance between the two treatment arms and a possible inter-subject variability of CPT-11 metabolism. Inter-patient variability of CPT-11 metabolism was previously reported [10], and such variability appears to be caused by inter-individual variability of carboxylesterase activity [4, 5], or glucuroconjugate activity correlated with UGT1A1 polymorphism [6]. In the present study, we could indeed observe a large inter-patient variability of CPT-11 catabolism, which is another good area for future investigation. This was not performed here since investigations into metabolic enzymes or genetic polymorphism with inter-patient comparison were not the specific aims of the present study. Here, we used intra-patient comparison to exclude inter-patient variability. As a result, we were able to clarify that BV has no effect on CPT-11 catabolism. Moreover, BV appeared to exert no effect on the conversion ratios of CPT-11 to SN-38 and SN-38 to SN-38G (Table 3). The explanation of the lacking pharmacokinetic interaction between BV and CPT-11 may be caused by different pathways of clearance: IgGs are cleared through Fc/Fc/Rn systems, whereas CPT-11 are primary enzymatically transformed in the liver [11, 12]. The analysis of PK parameters failed to provide any explanation for the observed supra-additive clinical efficacy of the CPT-11 and BV combination [2, 3]. The absence of PK interaction between CPT-11 and BV has been recognized to indicate the safety of this combination therapy for further clinical study and general practice.

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ORIGINAL PAPER

Adipocytokines and squamous cell carcinoma of the esophagus

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Abstract
Purpose Adipocytokines are adipocyte-secreted hormones associated with some malignancies. It has been reported that the impaired response of adipocytokines to body weight loss may play a role in the pathogenesis of cancer-induced cachexia. We investigated the association between adipocytokines with squamous cell carcinoma of the esophagus (SCCE).
Methods The levels of body mass index (BMI) and adiponectin, leptin, resistin, visfatin and C-peptide in the blood at diagnosis were measured in 117 SCCE patients and 117 age- and sex-matched controls. Logistic regression models were employed to estimate odds ratio. One-way analysis was performed to examine the prevalence of variables between two or more groups. A non-parametric Spearman correlation test was conducted to examine the associations between BMI and other variables.
Results Adiponectin and BMI levels were significantly lower, and resistin level was significantly higher in the patients on multivariate analysis ($P = 0.01$, <0.01 and <0.01 respectively). BMI gradually decreased with stage progression, and resistin level gradually increased with stage progression ($P < 0.01$ for both). The inverse correlation between BMI and adiponectin was comparatively strong in the controls, but was weak in the patients. Leptin showed comparatively strong correlation with BMI in the controls, but was weakly correlated in the patients. The correlation between BMI and resistin or C-peptide was demonstrated weakly only in the controls, and visfatin did not correlate with BMI.
Conclusions Resistin may be a biomarker for the progression of SCCE. In addition, the impaired responses to body weight loss of adiponectin and leptin in the patients with SCCE were suggested.

Keywords Esophageal cancer · Adiponectin · Leptin · Resistin · Visfatin · C-peptide

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Introduction

Patients with esophageal cancer tend to become cachexic with low body mass index (BMI) levels, because most of them present with gastrointestinal symptoms such as dysphagia, epigastralgia, nausea and anorexia. However, the carcinogenesis of adenocarcinoma of the gastroesophageal junction, of which the incidence has been increasing over the past three decades in Western countries, has been reported to be correlated with obesity (Calle and Thun 2004; Wolk et al. 2001). In Japan, the incidence of

squamous cell carcinoma of the esophagus (SCCE) remains at more than 90%, whereas that of adenocarcinoma of the gastroesophageal junction has remained very low. The risk of SSCE has never been reported to be correlated with obesity.

Adipocytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, type-1 plasminogen activator inhibitor, heparin-binding epidermal growth factor-like growth factor, hepatocyte growth factor, adiponectin, leptin, resistin, visfatin and apelin, are cytokines secreted by visceral adipose tissue, and they have recently been suggested to be associated with the risk of cancer at various sites (e.g., breast, prostate gland, endometrium and colorectum) as well as Barrett's esophagitis, which is the main risk factor for adenocarcinoma of the gastroesophageal junction (Rubenstein et al. 2008; Wei et al. 2005). There is as yet no case–control study investigating the correlation between adipocytokines and esophageal cancer.

On the other hand, adiponectin levels are inversely correlated with body weight, while leptin levels are directly associated with weight loss (Arita et al. 1999; Pannacciulli et al. 2003; Yang et al. 2001; Wolfe et al. 2004). Although the association between cancer-induced cachexia and adipocytokine level has been recently reported, the association between adipocytokine level and cancer-induced cachexia has not yet been fully elucidated (Kerem et al. 2008; Wolf et al. 2006) (Jamieson et al. 2004). In breast and colon cancer patients, no significant correlation was observed between adiponectin level and cachexia, and leptin level was significantly lower only in female cachexic patients than in female non-cachexic patients (Wolf et al. 2006). In gastric cancer patients, adiponectin, leptin and resistin levels were reportedly higher in cachexic patients than in non-cachexic patients (Kerem et al. 2008). These findings indicate that the impaired response of adipocytokines to body weight loss may play a role in the pathogenesis of cancer-induced cachexia.

We previously reported that resistin and visfatin may be biomarkers of gastric cancer, and adiponectin may have the possibility as a biomarker for the diagnosis of early stage gastric cancer (Nakajima et al. 2009). Moreover, the correlations between BMI and adiponectin were weaker in gastric cancer patients than that in the controls, and the correlation between BMI and leptin was observed both in the patients as well as in the controls. In the present study, we investigated the association between the levels of several blood adipocytokines and SCCE through a case–control study including BMI as a variable to speculate the importance of adipocytokines as biomarkers of SCCE and the association between cancer-induced cachexia and the changes in adipocytokines levels.

Materials and methods

Study population

Following the approval of the study protocol by the Institutional Review Board which conforms to the provisions of the Declaration of Helsinki in 1995, patients who underwent upper gastrointestinal (UGI) endoscopy at the National Cancer Center Hospital, Tokyo, from January 1999 to January 2007, who were considered to have no active malignancies except esophageal cancer, and whose blood samples at diagnosis before any treatments for SSCE could be obtained, were identified and invited to participate as patients or controls. Subjects pathologically and newly diagnosed as having SCCE by biopsy using UGI endoscopy and treated at our hospital for SCCE were identified as patients from the enrolled participants. Age- and sex-matched controls (1:1) diagnosed as being free from esophageal cancer by UGI endoscopy were identified from the enrolled participants. BMI at diagnosis was calculated on the basis of the data in medical records as follows: weight (kg)/height $(m)^2$. Clinical and pathological information for both groups was obtained from medical records. All subjects (patients and controls) provided an informed consent prior to the collection and analysis of blood samples.

Adipocytokines and C-peptide measurements

All blood samples were stored at −20°C until use. None of the samples were previously thawed. Blood levels of adiponectin, resistin, visfatin and C-peptide at diagnosis were measured by SRL Inc., Tokyo, Japan. Adiponectin was determined by enzyme-linked immunosorbent assay (ELISA) (Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan) with a sensitivity of 1.9 μg/ml, an intra-assay coefficient of variation of 3.5–5.1%, and an inter-assay coefficient of variation of 6.0–8.7%. Resistin was determined by ELISA (BioVender Laboratory Medicine, Brno, Czech Republic) with a sensitivity of 1.1 ng/ml, an intra-assay coefficient of variation of 2.8–3.4% and an inter-assay coefficient of variation of 5.1–6.9%. Leptin was measured using radioimmunoassay kits (LINCO Research, St Charles, MO, USA) with a sensitivity of 0.5 ng/ml, an intra-assay coefficient of variation of 3.4–8.3% and an inter-assay coefficient of variation of 3.0–6.2%. Visfatin was determined by ELISA (Adipo Gen, Seoul, Korea) with a sensitivity of 0.13 ng/ml, an intra-assay coefficient of variation of 4.4–10.4 % and an inter-assay coefficient of variation of 6.4–9.9%. C-peptide was determined by ELISA (Fujirebio, Tokyo, Japan) with a sensitivity of 0.04 ng/ml, an intra-assay coefficient of variation of 1.96–2.97% and an inter-assay coefficient of variation of 1.06–2.60%. Duplicate measurements were performed in a single experiment.

Table 1 Clinical characteristics of patients with esophageal cancer and controls

	Patient ($n = 117$)	Control ($n = 117$)	P value
Age (years)	63.6 ± 8.7	63.6 ± 8.8	0.63
Sex			
Female	30 (25.6%)	30 (25.6%)	
Male	87 (74.4%)	87 (74.4%)	1.00
Stage[a]			
0	21		
I	24		
II	24		
III	20		
IV	28		

Data are expressed as mean ± SD

[a] Japanese classification of esophageal carcinoma

Statistical analysis

The results of the comparison of clinical characteristics between patients and controls were evaluated by the χ^2 test for categorical variables and the two-sample t-test for continuous variables. Conditional logistic regression models were used for estimating odds ratio and 95% confidence interval (95% CI) to evaluate the association between each variable with SSCE. One-way analysis of variance was performed to examine the prevalence of each variable between tumor stage groups. Log transformations were conducted on variables prior to the analysis to achieve normal distribution. A non-parametric Spearman correlation test was conducted to examine the associations between BMI and other variables. Differences with $P \leq 0.05$ were considered statistically significant. All statistical analyses were carried out using the SAS system, version 9.1.3.

Results

Adipocytokine, C-peptide and BMI levels and SSCE risk

The clinical characteristics of the 117 patients and 117 controls are shown in Table 1. There was no significant difference in age and sex between the two groups. Adipocytokines, C-peptide and BMI levels of the patients and controls are shown in Table 2. The results of the univariate and multivariate logistic regression analyses are shown in Table 3. Adiponectin and BMI levels were significantly lower in the patients than in the controls on multivariate analysis ($P = 0.01$ and <0.01, respectively). On the other hand, resistin level was significantly higher in the patients than those in the controls on multivariate analysis ($P = < 0.01$).

Table 2 Adipocytokines, C-peptide and BMI levels in patients with esophageal cancer and controls

	Patient				Control			
	n	Median value	25th Quartile value	75th Quartile value	n	Median value	25th Quartile value	75th Quartile value
Adiponectin (μg/ml)	117	7.9	5.7	11.6	117	8.9	6.3	12.8
Resistin (ng/ml)	117	4.7	3.2	7.7	116	3.0	2.2	4.4
Leptin (ng/ml)	117	2.5	1.6	4.0	116	3.7	2.2	5.5
Visfatin (ng/ml)	117	2.6	1.1	6.7	117	1.4	0.8	2.6
C-peptide (ng/ml)	117	0.1	0.1	0.4	115	0.3	0.1	0.5
BMI (kg/m^2)	117	20.5	18.3	23.0	117	23.3	21.2	25.7

Table 3 Univariate and multivariate analyses of adipocytokines, C-peptide and BMI levels in patients with esophageal cancer and controls

Variable	Univariate analysis		Multivariate analysis	
	Odds ratio (95% confidence interval)	P value	Odds ratio (95% confidence interval)	P value
Adiponectin	0.617 (0.348–1.092)	0.10	0.296 (0.112–0.782)	0.01
Resistin	3.461 (1.997–5.998)	<0.01	3.428 (1.664–7.062)	<0.01
Leptin	0.364 (0.220–0.603)	<0.01	0.708 (0.317–1.581)	0.40
Visfatin	1.390 (1.124–1.719)	<0.01	0.990 (0.712–1.377)	0.95
C-peptide	0.739 (0.597–0.915)	<0.01	0.864 (0.619–1.206)	0.39
BMI (kg/m^2)	0.738 (0.658–0.828)	<0.01	0.738 (0.626–0.870)	<0.01

Table 4 Association between adiponectin, resistin, BMI and stage progression of esophageal cancer

Variable	Control		Stage 0		Stage 1		Stage 2		Stage 3		Stage 4		*P* value
	n		*n*		*n*		*n*		*n*		*n*		
Adiponectin*	117	2.2 ± 0.5	21	2.0 ± 0.5	24	2.3 ± 0.7	24	2.1 ± 0.5	20	1.9 ± 0.6	28	2.1 ± 0.5	0.25
Resistin*	117	1.2 ± 0.5	21	1.2 ± 0.6	24	1.4 ± 0.6	24	1.7 ± 0.5	20	1.6 ± 0.7	28	1.8 ± 0.7	<0.01
BMI (kg/m^2)	117	23.4 ± 3.3	21	21.8 ± 2.8	24	21.3 ± 3.5	24	19.9 ± 2.7	20	21.3 ± 3.3	28	18.8 ± 2.8	<0.01

Data are expressed as mean ± SD

*Log-transformed

Table 5 Spearman correlation coefficients between BMI and adipocytokines or C-peptide

Variable	BMI	
	Patient	Control
Adiponectin	−0.26	−0.45
Resistin	−0.14	0.25
Leptin	0.36	0.53
Visfatin	0.00	−0.10
C-peptide	0.18	0.20

Correlation of adiponectin, resistin and BMI levels with tumor stage

Linear contrast analysis was conducted to evaluate the correlation between adiponectin, resistin, BMI and tumor stage (Table 4). Because the ranges of adipocytokines and C-peptide levels were wide, we used the log-transformed values for them. BMI levels gradually decreased with stage progression ($P < 0.01$); in contrast, resistin level gradually increased with stage progression ($P < 0.01$).

Correlation between BMI levels and other variable levels

The Spearman correlation coefficients between BMI and other variables are shown in Table 5. The inverse correlation between BMI and adiponectin was comparatively strong in the controls but was weak in the patients. Leptin showed comparatively strong correlation with BMI in the controls, but was weakly correlated in the patients. The correlation between BMI and resistin or C-peptide was demonstrated weakly only in the controls, and visfatin did not correlate with BMI.

Discussion

In this case–control study, we demonstrated that the levels of adiponectin, resistin and BMI were significantly different between SCCE patients and controls. Resistin and BMI levels also significantly correlated with the stage progression of SCCE. The correlation between BMI and resistin was demonstrated weakly only in the controls, and resistin may be a biomarker of SCCE. An inverse correlation between adiponectin level and BMI was observed in the controls, and was weak in the patients. It indicates that the importance of adiponectin as a biomarker of SCCE cannot be completely evaluated in this study. In addition, the impaired responses to body weight loss of adiponectin and leptin in the patients with SCCE were suggested.

In case–control studies, adiponectin levels significantly decreased in patients with breast, endometrial, prostate, colon and gastric cancers and myelodysplastic syndrome (MDS) (Ishikawa et al. 2005; Wei et al. 2005; Dalamaga et al. 2008). Adiponectin suppresses the secretion of inflammatory cytokines such as TNF-α, and induces the secretion of anti-inflammatory cytokines such as IL-10 in the atherogenic process (Fantuzzi 2005; Kumada et al. 2004; Yokota et al. 2000). Furthermore, it has been reported to inhibit tumor growth by suppressing angiogenesis in vitro and in vivo (Wang et al. 2005). These data suggest that the decreased systemic level of adiponectin may indicate its decreased protective physiological function against inflammation or angiogenesis. The association between leptin level and the risk of cancer has remained controversial in breast, prostate and colon cancers (Chia et al. 2007; Stattin et al. 2004). On the other hand, the normal response of adiponectin or leptin to body weight change was reported as follows: adiponectin level inversely correlates with body weight, and leptin level decreases in response to body weight loss (Arita et al. 1999; Pannacciulli et al. 2003; Wolfe et al. 2004). In the cachexic status, however, these responses might be impaired, in which case adiponectin cannot respond to body weight loss by increasing secretion, and leptin cannot respond to body weight loss by decreasing secretion (Jamieson et al. 2004; Kerem et al. 2008; Wolf et al. 2006). Our results showed the inverse correlation between adiponectin and SSCE. However, an inverse correlation between adiponectin and BMI was observed, and we could not evaluate the importance of adiponectin as a biomarker of SCCE in this study. The correlation between BMI and leptin also existed. Both these