study. With this population, response rates typically have not exceeded 10% in patients treated with 5-fluorouracil (5-FU); therefore, a response rate of at least 15% in our study would suggest a potential benefit.

Our goal was to enroll 40 eligible patients. If no response occurred in the first 18 patients, accrual was terminated because the chance of a 15% response rate was only 5.3%. If the response rate was 15%, the statistical power (the probability of a 5% response rate) would be 73% with type I error of 5% (one-sided). For a response rate of 17.5%, the statistical power would be 85%, and the statistical power would be 92% for a response rate of 20%.

All time-to-event measures were calculated using the Kaplan–Meier method.

Results

Patient characteristics and disposition

From October 2001 to September 2003, 21 males and 19 females, with a median age of 61 years (range 33–73 years), were enrolled. Table 1 shows the baseline patient characteristics. Twenty-three patients (57.5%) had no prior therapy, and 17 (42.5%) relapsed after resection for primary lesion. The major metastatic lesions were the abdominal lymph nodes (67.5%) and liver (55.0%). Prior to the initiation of study treatment, obstructive jaundice was palliated with percutaneous transhepatic catheter placement (11 patients) or endobiliary stent placement (3 patients).

The reasons for the treatment discontinuation included progressive disease (34 patients), elevated blood pressure associated with worsening of renal function (one patient), hemolytic uremic syndrome (one patient), blood bilirubin increased with progressive disease (one patient), relapse of pre-existing schizophrenia (one patient), patient's refusal due to nausea/vomiting (one patient), and general fatigue (one patient).

Table 1 Baseline patient characteristics ($n=40$)

Characteristic	
Gender, *n* (%)	
Male	21 (52.5)
Female	19 (47.5)
Age, years	
Median (range)	61 (33–73)
ECOG performance status	
0	24 (60.0)
1	16 (40.0)
Primary lesion	
Extrahepatic bile duct	12 (30.0)
Gallbladder	22 (55.0)
Ampulla of Vater	6 (15.0)
CA19–9, *n* (U/ml)	
Median (range)	448.6 (1–77,820)
CEA, *n* (ng/ml)	
Median (range)	10.9 (0.5–1,790)
Metastatic sites, *n* (%)	
Abdominal lymph nodes	27 (67.5)
Liver	22 (55.0)
Peritoneum	4 (10.0)
Lung	2 (5.0)
Bone	1 (2.5)

ECOG Eastern Cooperative Oncology Group; *CA19–9* carbohydrate antigen 19–9; *CEA* carcinoembryonic antigen

Efficacy

All 40 patients were evaluated for efficacy and according to WHO criteria, seven patients achieved a partial response for an overall response rate of 17.5% (95% CI, 7.3–32.8%). The median duration of the response was 9.4 months (range, 2.6–9.4 months). Fifteen patients (37.5%) had stable disease, and 17 patients (42.5%) had progressive disease. Tumor response was not determined in one patient because she was transferred to another hospital before response evaluation. The serum CA 19–9 level was reduced by less than half in 11 (33%) of 33 patients who had a pretreatment level of above upper normal limit, and the CEA level was reduced by less than half in 6 (24%) of 25 patients. Of the 11 patients whose CA 19–9 level was reduced, 4 (36%) showed a partial response. Five (83%) of the six patients with the CEA response achieved a partial response.

At the time of analysis, 35 of 40 patients had died of cancer and two of five patients lived longer than 24 months after the initial administration of gemcitabine. The median progression-free interval was 2.6 months (95% CI, 1.7–3.8 months), and the median survival time was 7.6 months (95% CI, 5.4–9.3 months) (Fig. 1). The 1-year survival rate was 25.0%.

Toxicity

All 40 patients were evaluable for toxicity (Table 2). No toxic deaths occurred. Hematologic toxicity was reversible and manageable. Patients reported grade 3/4 neutropenia (30.0%), leukopenia (12.5%), and anemia (10.0%). Three patients had red blood cell transfusions due to hemolytic uremic syndrome, hemorrhagic shock, and anemia. No grade 3/4 thrombocytopenia was reported. Although two patients were treated with G-CSFs, there was no febrile neutropenia.

The most common nonhematologic toxicities, grades 1–4 were nausea (52.5%) and anorexia (52.5%), but only four patients (10%) required intravenous infusion due to these toxicities. The most common grade 3/4 nonhematologic toxicities were elevated ALT (15.0%) and elevated γ-glutamyltransferase (γ-GTP) (12.5%). Grade 4 elevated γ-GTP was observed in one patient, which was considered to be gemcitabine-related because the level returned to normal after treatment discontinuation. The patient, who had grade 3 uremia, grade 2 serum creatinine elevation, and grade 2 thrombocytopenia, was diagnosed with grade 4 hemolytic uremic syndrome and also recovered from these toxicities by

transfusion without dialysis after discontinuing gemcitabine. In another patient on day 25 of cycle 1, hemorrhagic shock occurred following unexpected hematemesis, which was unlikely to be gemcitabine related. Endoscopic examination showed acute gastric mucosal lesions, and prescribed nonsteroidal anti-inflammatory drugs to control abdominal pain were suspected to be the cause of hemorrhagic shock.

Dose intensity

A median of three cycles was administered (range, 1–14). Eleven patients (27.5%) completed one cycle; eight patients (20.0%) completed two cycles; and five patients (12.5%) completed three cycles. The planned mean dose intensity of gemcitabine was 750 mg/m^2; however, the actual mean dose intensity of gemcitabine was 688.7 mg/m^2. Thus, the dose intensity was 91.8% for gemcitabine. Of the 476 planned infusions, 37 dose omissions (7.8%) occurred, mainly due to neutropenia. There were no dose reductions.

Discussion

The vast majority of patients with biliary tract cancer are candidates for chemotherapy; however, chemotherapy for biliary tract cancer currently has only limited value in clinical practice. 5-FU is the mainstay of palliative chemotherapy, although response rates range from 0 to 13% in phase II trials [6, 11, 39]. It is generally accepted that combinations with 5-FU have little superiority over single-agent 5-FU, and the considerable toxicity often outweighs the benefit for the patients [11, 39]. Except for gemcitabine, no individual agent has shown a reproducible response rate over 15% [1, 12, 19, 29, 31, 33, 37]. Therefore, new agents need to be developed for truly effective chemotherapeutic regimens against this disease.

In a prospective randomized trial [4], gemcitabine is the only agent showing significant efficacy in respect to survival prolongation and symptom relief for patients with advanced pancreatic cancer; these results prompted trials for biliary tract cancer, which, to some extent, shares embryological and clinical features with pancreatic cancer. Several early-phase studies of single-agent gemcitabine at doses of 1,000–2,200 mg/m^2 have reported response rates of 8–60%, and median survival durations ranging from 6.5 to 11.5 months. [3, 7, 8, 14, 21, 24, 34, 35].

In our trial, gemcitabine 1,000 mg/m^2 was administered for 3 weeks with 1 week of rest; this schedule is currently approved in Japan for non-small-cell lung cancer and pancreatic cancer and is considered to be a standard regimen worldwide. Our overall response rate of 17.5% appeared to be comparable to previous trials with gemcitabine or other combination regimens and appeared near the highest results in single-agent therapy. In recent phase II trials of various single agents, responses were 8% in a study with cisplatin [29], 0% in paclitaxel [19], 0–25% in docetaxel [2, 31, 33], 11% in irinotecan [12], and 19% in capecitabine [23]. Our median overall survival of 7.6 months was also comparable to other trials of single-agent therapy, which ranged from 4.5 to 8.0 months [2, 12, 19, 23, 29, 31, 33, 37], and for combination therapies, which ranged from 5.0 to 14.0 months [5, 9, 10, 15, 18, 20, 26, 28, 32, 35, 36, 38]. However, it seemed to be longer when compared with other phase II trials for Japanese patients with advanced or metastatic biliary tract cancer, which was 5.3 months in uracil/tegafur, 5.9 months in cisplatin/

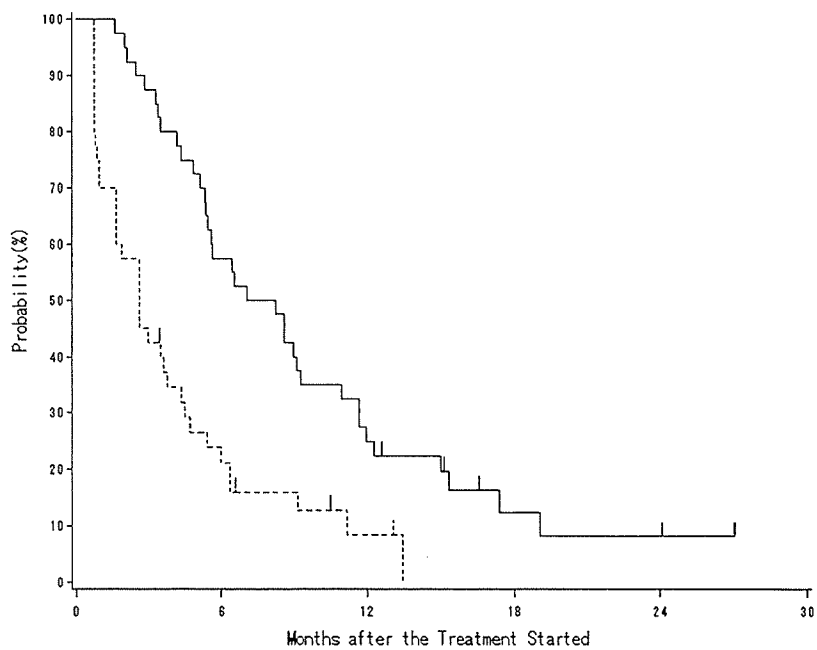

Fig. 1 Progression-free survival (*dashed line*) and overall survival (*solid line*) curves of patients with advanced biliary tract cancer receiving systemic chemotherapy with gemcitabine

Table 2 Adverse drug reaction

Adverse drug reaction	Grade 3		Grade 4	
	n	(%)	*n*	(%)
Hematologic toxicities				
Neutropenia	10	25.0	2	5.0
Leukopenia	5	12.5	0	0.0
Anemia	3	7.5	1	2.5
Thrombocytopenia	0	0.0	0	0.0
Nonhematologic toxicities				
Elevated ALT	6	15.0	0	0.0
Elevated γ-GTP	4	10.0	1	2.5
Elevated AST	2	5.0	0	0.0
Decreased serum sodium	2	5.0	0	0.0
Increased serum ALP	2	5.0	0	0.0
Urinary occult blood positive	1	2.5	0	0.0
Increased serum bilirubin increased	0	0.0	0	0.0
Increased serum creatinine	0	0.0	0	0.0
Proteinuria	0	0.0	0	0.0
Hematuria	0	0.0	0	0.0
Hemolytic uremic syndrome	0	0.0	1	2.5
Constipation	3	7.5	0	0.0
Vomiting	3	7.5	0	0.0
Nausea	2	5.0	0	0.0
Hematemesis	0	0.0	1	2.5
Diarrhoea	0	0.0	0	0.0
Stomatitis	0	0.0	0	0.0
Fatigue	0	0.0	0	0.0
Edema	0	0.0	0	0.0
Pyrexia	0	0.0	0	0.0
Biliary tract infection	1	2.5	0	0.0
Anorexia/Appetite impared	3	7.5	1	2.5
Rash	1	2.5	0	0.0
Alopecia	0	0.0	0	0.0
Hypertension	1	2.5	0	0.0
Hemorrhagic shock	0	0.0	1	2.5

ALT Alanine aminotransferase, *γ-GTP* γ-glutamyltransferase, *AST* aspartate aminotransferase, *ALP* alkaline phosphatase

epirubicin/5-FU, and 5.5 months in a study with cisplatin [18, 26, 29].

The toxicity profile in our study was generally acceptable. The major toxicities were myelosuppression; the incidences of grade 3/4 toxicities were 30.0% in neutropenia, 12.5% in leukopenia, and 10.0% in anemia. However, grade 4 toxicities were infrequent, and neither febrile neutropenia nor treatment-related deaths were observed. The toxicity profile in our study was consistent with past studies using gemcitabine in other tumors. For patients treated with cisplatin, epirubicin, and 5-FU [26], high incidences of grade 3/4 neutropenia (76.0%), leukopenia (59.0%), and death due to treatment-related sepsis 5.0% occurred despite a response rate (19%) similar to that in our study. There was only one episode of cholangitis in this study, although patients with biliary tract cancer are at high-risk for cholangitis, and sometimes severe sepsis occurs, which is derived from cholangitis during chemotherapy [26]. Transient elevations of hepatic enzymes have been reported in gemcitabine therapy for both pancreatic and biliary tract cancer; liver function may be easily affected by cholestasis due to existence of primary and/or metastatic tumors.

One patient developed hemolytic uremic syndrome, which was considered to be a manifestation of thrombotic microangiopathy, although gemcitabine-associated thrombotic microangiopathy is believed to be very rare, with estimated incidences of 0.008–0.31% [13, 17]. The event in this patient seemed to be a treatment-related adverse reaction; however, the patient recovered from hemolytic uremic syndrome without hemodialysis after discontinuation of gemcitabine. Grade 4 anemia was observed in one patient, who suffered grade 4 hematemesis and hemorrhagic shock. This was unlikely to be related to gemcitabine because no thrombocytopenia was observed in this patient. Also, upper gastrointestinal endoscopy revealed acute gastric mucosal lesions as the origin of the bleeding, which seemed to be related to prescribed non-steroidal anti-inflammatory drugs.

Our study was conducted among the largest group of patients with biliary tract cancer to date. In our study, gemcitabine was administered to patients who had biliary stent insertion or percutaneous biliary drainage, and no particular drug-related toxicity was observed in these patients. The result of our study is promising for patients with biliary tract cancer.

In conclusion, chemotherapy with single-agent gemcitabine was feasible and appeared to show efficacy in advanced or metastatic biliary tract cancer. Gemcitabine may provide a more favorable prognosis in patients with this disease compared to other chemotherapeutic regimes or best supportive care.

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Appendix

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SNP Communications

Novel Single Nucleotide Polymorphism of UGT1A7 Gene in Japanese

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Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: We sequenced exon 1 of the UDP-glucuronosyltransferase (UGT) *1A7* gene from 52 Japanese cancer patients. Four single nucleotide polymorphisms (SNPs) were found. Three of them caused *UGT1A7*2* and *UGT1A7*3*. A novel SNP (98973G>C) causing amino acid substitution ($Ser^{141}Cys$) was found. The sequence is as follows: SNP, 050824FujitaK002; GENE NAME, UGT1A7; ACCESSION NUMBER, AF297093; LENGTH, 25 bases; 5′-TAAAGGAGAGTTG/CTTTTGATGC-AGT-3′. One out of 52 cancer patients was heterozygous for the variant allele, resulting in the allele frequency of 0.96%. The patient did not possess *UGT1A7*2* or *UGT1A7*3*.

Key words: ***UGT1A7*; novel SNP; Japanese; Irinotecan**

Introduction

UDP-glucuronosyltransferase (UGT) is one of phase II drug-metabolizing enzymes that catalyzes the glucuronidation of a wide variety of endogenous and exogenous compounds, including drugs, carcinogens, and other xenobiotics. The UGT superfamily is composed of families, and two of the families (UGT1 and UGT2) have the ability to catalyze the glucuronidation of foreign chemicals.[1] The *UGT1A7* gene located on chromosome 2q37 is expressed exclusively in the oropharynx, esophagus, stomach and pancreas,[2-6] but is absent from the liver.[7] Cloning and characterization of the *UGT1A7* gene product revealed the glucuronidation of known carcinogens, which included tobacco smoke carcinogens.[8,9] Chemicals with phenolic, anthraquinone, flavone, coumarin and naphthol structures are substrates for *UGT1A7*.[7] *UGT1A7* is also known to be involved in the glucuronidation of an active metabolite of an anticancer drug irinotecan (SN-38).[10] Various single nucleotide polymorphisms (SNPs) have been identified in the *UGT1A7* gene so far.[6,10-13] *UGT1A7*3* has been known to cause the reduced capacity to metabolize SN-38, whereas the product of *UGT1A7*2* gene showed higher activity toward SN-38 than that of *UGT1A7*1*.[10,13] The effects of these polymorphisms on the irinotecan toxicity in humans seem somewhat contradictory. Carlini *et al.*[11] claimed that the low *UGT1A7* activity was related to the little toxicity of irinotecan, whereas Ando *et al.*[14] demonstrated no relationship between the reduced *UGT1A7* activity and the irinotecan toxicity.

In the present study, the exon 1 of *UGT1A7* gene from 52 Japanese cancer patients treated with irinotecan was sequenced, and found a novel SNP causing amino acid substitution.

Materials and Methods

Patients: Fifty-two Japanese cancer patients (32 colorectal cancer, 15 gastric cancer, 5 others) who received various regimens of irinotecan-containing chemotherapy or irinotecan monotherapy from June 2003 to August 2005 were studied. All of patients gave informed consent in writing for their peripheral blood samples and medical information to be used for the research. This study was approved by the Institutional Review Board of Saitama Medical School.

Human DNA samples: Genomic DNA was extracted from 200 μL of peripheral blood of 52 Japanese

On September 30, 2005, the variation was not found on the UDP-glucuronosyltransferase homepage (http://som.flinders.edu.au/FUSA/ClinPharm/UGT/), the Japanese Single Nucleotide Polymorphism (JSNP) (http://snp.ims.u-tokyo.ac.jp/index_ja.html) or dbSNP in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/).

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cancer patients, which had been stored at −80°C until analysis, by using QIAamp Blood Kit (QIAGEN GmbH, Hilden, Germany).

PCR conditions and DNA sequencing: Exon 1 of the *UGT1A7* gene was analyzed by direct sequencing of a PCR product by the method of Carlini *et al.*[11], with minor modifications. Briefly, the reaction mixture for PCR consisted of 2.5 mM $MgCl_2$ and 1.25 unit of AmpliTaq Gold polymerase (Perkin-Elmer, Foster City, CA) in a final volume of 50 μL. The sequence of the complete *UGT1A7* gene described in the GeneBank (AF297093) was used as a reference.

Two polymorphisms of *UGT1A1* gene (*UGT1A1*6* and *UGT1A1*27*) were determined by PCR-RFLP method as described by Ando *et al.*,[15] with minor modifications. Briefly, the first and the second PCRs were performed with AmpliTaq Gold. The reaction mixture for the first PCR consisted of 2 mM $MgCl_2$ and 1.25 unit of AmpliTaq Gold polymerase in a final volume of 50 μL. The second PCR was carried out with the reaction mixture consisted of 1.5 mM $MgCl_2$ and 1.25 unit of AmpliTaq Gold polymerase in a final volume of 50 μL. The PCR conditions for the second PCR were: 95°C 15 min followed by 25 cycles of 94°C for 30 s, 55°C for 40 s and 72°C for 40 s. RFLP analyses for *UGT1A1*6* and *UGT1A1*27* were performed with *Msp* I (Takara, Otsu, Japan) and *Bsr* I (New England Biolabs, Ipswich, MA), respectively. The TATA box polymorphism (*UGT1A1*28*) was determined by the direct sequencing described by Ando *et al.*,[15] with minor modifications. Briefly, the reaction mixture for the first PCR consisted of 1 mM $MgCl_2$ and 1.25 unit of AmpliTaq Gold polymerase in a final volume of 50 μL. The PCR conditions for the first PCR were: 95°C 15 min followed by 30 cycles of 95°C 30 s, 58°C 40 s and 72°C 40 s.

Treatments: Irinotecan as a monotherapy was given weekly at a dose of 100 mg/m^2 for the first 3 weeks of each 4-week cycle,[16] or biweekly at a dose of 150 mg/m^2 [17] until the disease showed progression or intolerable toxicity occurred. As combination chemotherapy, a 100 mg/m^2 irinotecan was administered with the bolus 5-fluorouracil (FU) 500 mg/m^2 and leucovorin (LV) 10 mg/m^2 (l isomer form) weekly for the first 4 weeks of each 6-weeks cycle (IFL regimen).[18] The FOLFIRI regimen administered at 2-week intervals comprised irinotecan at escalating doses from 150 to 180 mg/m^2 and LV 200 mg/m^2 administered over 2 hours followed by FU 400 mg/m^2 as a bolus injection and FU 2,400 mg/m^2 as a 46-hour continuous infusion.[19] As the other regimen repeated every 4 week (IP regimen), irinotecan at doses from 50 to 70 mg/m^2 was followed 2 hours later by a 120-minute infusion of cisplatin 80 mg/m^2 with adequate hydration on day 1, and the same dose of irinotecan was repeated on day 15.[20] In each regimen, irinotecan at doses from 50 mg/m^2 to 180 mg/m^2 dissolved in 250 mL of 5% dextrose solutions was infused over 90 minutes.

Pharmacokinetic analysis: Blood sampling for pharmacokinetic analysis was typically performed at their first courses. The blood samples for the analysis were taken from the arm opposite the infusion site at the beginning of irinotecan infusion and 0, 0.25, 0.5, 1, 2, 4, 8, and 24 hours after the end of the infusion. Plasma was immediately obtained by centrifugation of the blood samples and stored at −80°C until analysis.

Total (lactone and carboxylate) plasma concentrations of irinotecan, SN-38 and SN-38 glucuronide (SN-38G) were analyzed at the institution, using a reverse-phase HPLC method. A 150 μL of plasma sample was mixed with 300 μL of methanol, 5% perchloric acid (50:50, v/v) and camptothecin as an internal standard by a vortex mixer. The 200 μL of supernatant obtained by centrifugation of the mixture at 15,000 rpm for 10 min was injected into a HPLC system (Hitachi model 7000 series, Hitachi, Tokyo, Japan) equipped with a TSK-gel ODS-120T analytical column (4.6×250 mm; 4 μm; TOSOH, Tokyo, Japan), and separated at 40°C, at a flow rate of 1.0 mL/min to quantify the total (lactone and carboxylate) plasma concentrations of irinotecan, SN-38 and SN-38G. The mobile phase consisted of 75 mM ammonium acetate (pH 4.75) for solvent A and acetonitrile for solvent B: a 20-minute run of a linear gradient of 85% to 65% of solvent A. Lower limit of quantification for irinotecan was 5 ng/mL, and those for SN-38 and SN-38G were 0.5 ng/mL. The intra- and inter-assay coefficient variations for irinotecan and the metabolites were under 10%.

Area under the time versus concentration curve (AUC, μmol·h/l) from the beginning of the infusion to the last sampling was calculated by the linear trapezoidal rule, using a computer program, WinNonlin version 4.01 software (Pharsight Corporation, Mountain View, Calf).

Results and Discussion

We found a following novel SNP:
SNP, 050824Fujita002; GENE NAME, UGT1A7; ACCESSION NUMBER, AF297093; LENGTH, 25 bases; 5′-TAAAGGAGAGTTG/CTTTTGATGCAGT-3′. The SNP was 98973G>C in exon 1 of *UGT1A7* (**Fig. 1**), resulting in the amino acid substitution ($Ser^{141}Cys$). This novel SNP was found in one Japanese colorectal cancer patient with a heterozygousity. Thus, the allele frequency was calculated to be 0.96%. The patient possessing the novel SNP did not harbor *UGT1A7*2* or *UGT1A7*3* variant in exon 1, suggesting the no linkage among these SNPs.

Although functional significance of this SNP has not been known, it may cause functional reduction of

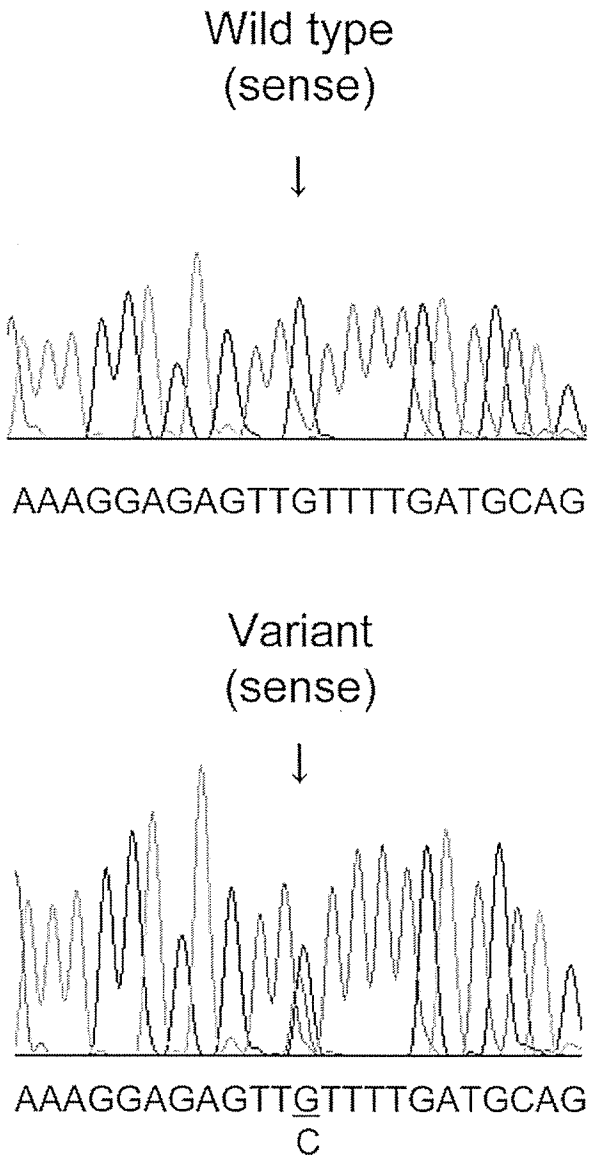

Fig. 1. Nucleotide sequences of *UGT1A7* containing novel variant 050824FujitaK002 (98973G>C) in exon 1.
Arrows indicate the positions of the nucleotide change.

UGT1A7 because the amino acid of 141 is located in the putative substrate-binding domain of the enzyme (between Ser^{61} and Phe^{190}).[21] The idea is considered to be supported by the fact that the decrease of the enzymatic activity of *UGT1A7* by the known amino acid substitutions in the putative substrate-binding domain (*UGT1A7.3*).

UGT1A7 plays a role in the glucuronidation of the active metabolite of irinotecan (SN-38).[10] Therefore, patient with reduced activity of UGT1A7 may have decreased ability to detoxify the SN-38. Besides *UGT1A7*, *UGT1A1* is known to be responsible for the detoxification of SN-38,[10] and the polymorphisms in the *UGT1A1* gene such as *UGT1A1*28*, *UGT1A1*6* and *UGT1A1*27* have been demonstrated to be related to the enhanced toxicity of irinotecan such as diarrhea and neutropenia.[15] The analysis of the *UGT1A1* polymorphisms for the patient with the novel SNP revealed that the patient harbored the wild type of *UGT1A1* gene. Therefore, to clarify the effects of the novel SNP in *UGT1A7* gene on the glucuronidation capacity for SN-38, pharmacokinetics in SN-38 and SN-38G were compared between the patient with the novel SNP (not possessing *UGT1A7*2* or *UGT1A7*3* and *UGT1A1*28*, *UGT1A1*6* or *UGT1A1*27*) and patients without all of these SNPs in *UGT1A1* and *UGT1A7* genes. According to the genetic analysis, 16 patients possessed the wild type of *UGT1A1* and *UGT1A7*. The AUC ratio of SN-38 to SN-38G seen in the patient with the novel SNP (0.42) was not necessarily higher than those observed in the 16 patients (0.38 to 0.57; quartile range). In addition, the patient possessing the novel SNP did not show severe toxicity by the irinotecan treatment. Accordingly, heterozygosity of the novel SNP appeared not to affect the pharmacokinetics of irinotecan metabolites and the toxicity of irinotecan. The functional significance of the novel SNP in the *UGT1A7* gene is needed to be examined in the future.

In conclusion, we found a novel nonsynonymous mutation 98973G>C in exon 1 of *UGT1A7* in a DNA sample from a Japanese colorectal cancer patient. The SNP caused the amino acid substitution ($Ser^{141}Cys$).

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Drug Metab. Pharmacokinet. 21 (1): *SNP5* (79)-*SNP7* (81) (2006).

SNP Communication

Novel Single Nucleotide Polymorphism of UGT1A9 Gene in Japanese

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Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: We sequenced from 5′-franking region to intron 1 (to 337 bp downstream from exon 1) of the UDP-glucuronosyltransferase (UGT) *1A9* gene prepared from 55 Japanese cancer patients. Seven single nucleotide polymorphisms (SNPs) were found. Two of them were *UGT1A9* -118(T)$_n$ (n=10) and *UGT1A9*5*, and four were reported SNPs in intron 1 of *UGT1A9* gene (89540C>T, 89549G>A, 89616T>A and 89710A>C). A novel SNP (89587T>C) was found. The sequence is as follows: SNP, 050824FujitaK001; GENE NAME, UGT1A9; ACCESSION NUMBER, AF297093; LENGTH, 25 bases; 5′-CCTTCTTGAAGAT/CATGTATTTATAA-3′. Two patients were heterozygous for the mutant allele, resulting in the allele frequency of 1.82%.

Key words: UGT1A9; novel SNP; Japanese

Introduction

UDP-glucuronosyltransferase (UGT) is one of phase II drug-metabolizing enzymes that catalyzes the glucuronidation of a wide variety of endogenous and exogenous compounds, including drugs, carcinogens, and other xenobiotics. The UGT superfamily is composed of families, and two of the families (UGT1 and UGT2) have the ability to catalyze the glucuronidation of foreign chemicals.[1] The *UGT1A9* gene located on chromosome 2q37 is expressed in the liver, kidney, small intestine, colon, and reproductive organs such as testis and ovary.[2-4] Cloning and characterization of the *UGT1A9* gene product demonstrated the conjugation of endogenous estrogenic and thyroid hormones,[3,5] as well as various xenobiotics such as phenols,[6] acetaminophen,[7] and an active metabolite of irinotecan (SN-38).[8] So far, various single nucleotide polymorphisms (SNPs) have been identified in the *UGT1A9* gene.[9-13] *UGT1A9*3* and *UGT1A9*5* have been known to cause the reduced ability to metabolize SN-38.[9,11] The SNP in the 5′-franking region of the *UGT1A9* gene (-118(T)$_n$ (n=10)) (based on assigning the 'A' in the 'ATG' translation start codon as +1) has been reported to be related to the enhanced transcriptional activity of the gene.[12]

In the present study, 5′-franking region to intron 1 (to 337 bp downstream from exon 1) of the *UGT1A9* gene from 55 Japanese cancer patients treated with irinotecan was sequenced, and found a novel SNP in intron 1.

On September 30, 2005, the variation was not found on the UDP-glucuronosyltransferase homepage (http://som.flinders.edu.au/FUSA/ClinPharm/UGT/), the Japanese Single Nucleotide Polymorphism (JSNP) (http://snp.ims.u-tokyo.ac.jp/index_ja.html) or dbSNP in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/).

Materials and Methods

Patients: Fifty-five Japanese cancer patients (33 colorectal cancer, 15 gastric cancer, 3 ovarian cancer and 4 others) who received various regimens of irinotecan-containing chemotherapy or irinotecan monotherapy from June 2003 to August 2005 were studied. All of patients gave informed consent in writing for their peripheral blood samples and medical information to be used for the research. This study was approved by the Institutional Review Board of Saitama Medical School.

Human DNA samples: Genomic DNA was extracted from 200 μL of peripheral blood of 55 Japanese cancer patients, which had been stored at −80°C until analysis, using QIAamp Blood Kit (QIAGEN GmbH, Hilden, Germany).

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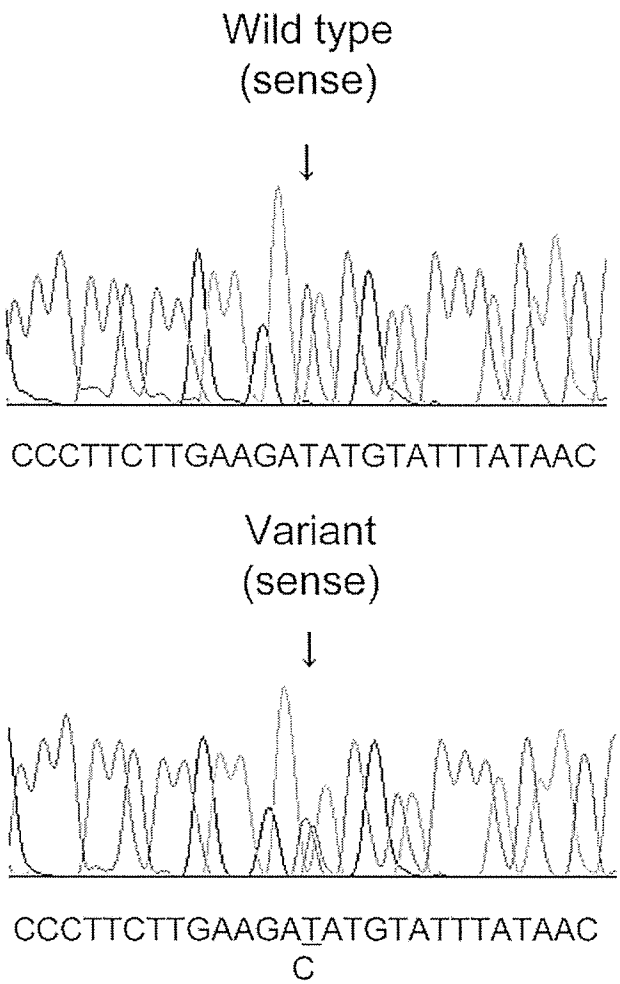

Fig. 1. Nucleotide sequences of *UGT1A9* containing novel variant 050824FujitaK001 (89587T>C) in intron 1.
Arrows indicate the positions of the nucleotide change.

PCR conditions and DNA sequencing: A 1.46 kb fragment (from 88271 to 89734) including 5′-franking region, exon 1 and intron 1 (to 337 bp downstream from exon 1) of *UGT1A9* gene was amplified by PCR, according to the method of Carlini *et al.*,[14] with minor modifications. Briefly, the reaction mixture consisted of 2.5 mM $MgCl_2$ and 1.25 unit of AmpliTaq Gold polymerase (Perkin-Elmer, Foster City, CA) in a final volume of 50 μL. Polymorphisms were detected by direct sequencing as described previously.[14] The sequence of the complete *UGT1A9* gene described in the GeneBank (AF297093) was used as a reference.

Results and Discussion

We found a following novel SNP: SNP, 050824Fujita001; GENE NAME, UGT1A9; ACCESSION NUMBER, AF297093; LENGTH, 25 bases; 5′-CCTTCTTGAAGAT/CATGTATTTATAA-3′. The SNP was 89587T>C in intron 1 of the *UGT1A9* gene (**Fig. 1**). This novel SNP was heterozugously found in two Japanese cancer patients (colorectal cancer and ovarian cancer). Thus, the allele frequency was calculated to be 1.82% (**Table 1**). Besides the novel SNP, a known SNP was observed in the 5′-franking region (*UGT1A9* -118$(T)_n$ (n=10)). The allele frequency of the SNP calculated in this study was consistent with that observed in the previous study.[12] The other was seen in exon 1 (*UGT1A9*5*) and four were detected in intron 1. We did not find the *UGT1A9*2*, *UGT1A9*3* or *UGT1A9*4* polymorphisms, probably because the low allele frequency of these SNPs in Japanese.[10] In Caucasians, *UGT1A9*3* was reported to be found with the allele frequencies of 0.633 to 3.6%,[9,15] whereas *UGT1A9*5* was demonstrated not to be observed.[15] On the other hand, *UGT1A9*2* was found in Africans with the allele frequency of 2.5%.[9] These results suggest the ethnic difference in the distribution of *UGT1A9* polymorphisms. Intronic SNPs, 89616T>A and 89710A>C, were completely linked. Furthermore, these SNPs were highly related to the *UGT1A9* -118$(T)_n$ (n=10) polymorphism. One of patients with the novel SNP was also heterozygous for *UGT1A9* -118$(T)_n$ (n=10), 89616T>A and 89710A>C. No severe toxicity, diarrhea or neutropenia, was not observed in these patients. At present, the functional significance of this SNP is not known. Further studies including the analysis of linkage between the novel SNP and other SNPs are needed to clarify the importance of the novel SNP.

Table 1. Allele frequency of polymorphisms in *UGT1A9* seen in Japanese cancer patients

		Allele number	Frequency
5′-Franking region	*UGT1A9* -118(T)n (n=10)	70	63.6
Exon1	*UGT1A9*5*	1	0.91
Intron 1	89540C>T	1	0.91
	89549G>A	13	11.8
	89587T>C	2	1.82
	89616T>A	66	60.0
	89710A>C	66	60.0

In conclusion, we found a novel mutation 89587T>C in intron 1 of *UGT1A9* in DNA samples from two Japanese colorectal and ovarian cancer patients.

Acknowledgment: We thank Ms Yuko Akiyama for technical help in the sequence analysis.
This study was supported in part by a Grant-in-Aid from the Ministry of Health and Welfare of Japan (13-10).

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Pharmacogenetic impact of polymorphisms in the coding region of the *UGT1A1* gene on SN-38 glucuronidation in Japanese patients with cancer

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Pharmacogenetic testing for UDP-glucuronosyltransferase (*UGT*) *1A1*28*, a promoter variant of the *UGT1A1* gene, is now carried out clinically to estimate the risk of irinotecan-associated toxicity. We studied the clinical significance of *UGT1A1*6* and *UGT1A1*27*, two variants in exon 1 of the *UGT1A1* gene that are found mainly in Asians. The study group comprised 46 Japanese patients who received various regimens of chemotherapy including irinotecan at doses from 50 to 180 mg/m^2. Pharmacogenetic relationships were explored between the *UGT1A1* genotype and the ratio of the area under the plasma concentration–time curve (AUC) of the active metabolite of irinotecan (SN-38) to that of SN-38 glucuronide (SN-38G), used as a surrogate for UGT1A1 activity ($AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$). No patient was homozygous for *UGT1A1*28*, and none had *UGT1A1*27*. Two were heterozygous for *UGT1A1*28*. Two were homozygous and 15 heterozygous for *UGT1A1*6*, all of whom were wild type with respect to *UGT1A1*28*. Two patients were simultaneously heterozygous for *UGT1A1*28* and *UGT1A1*6*, present on different chromosomes. The other 25 patients had none of the variants studied. The two patients simultaneously heterozygous for *UGT1A1*28* and *UGT1A1*6* and the two patients homozygous for *UGT1A1*6* had significantly higher $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ ratios than the others ($P = 0.0039$). Concurrence of *UGT1A1*28* and *UGT1A1*6*, even when heterozygous, altered the disposition of irinotecan remarkably, potentially increasing susceptibility to toxicity. Patients homozygous for *UGT1A1*6* should also be carefully monitored. *UGT1A1* polymorphisms in the coding region of the *UGT1A1* gene should be genotyped in addition to testing for *UGT1A1*28* to more accurately predict irinotecan-related toxicity, at least in Asian patients. (*Cancer Sci* 2006; 97: 1255–1259)

Irinotecan is a camptothecin analog with potent antitumor activity resulting from the inhibition of topoisomerase. This anticancer drug is now used widely to treat colorectal, lung and other types of cancer.[1,2] Dose-limiting toxicity of irinotecan includes severe leukopenia, neutropenia and diarrhea.[3,4] Post-marketing studies of approximately 14 000 patients with cancer who received irinotecan in Japan have estimated that the incidence of grade 3 or higher leukopenia is 23.8% for irinotecan monotherapy and 38.3% for irinotecan combined with other drugs, whereas that of grade 3 or higher diarrhea is approximately 10%, regardless of regimen.[3]

Genetic polymorphisms of UDP-glucuronosyltransferase (*UGT*) *1A1*, a key metabolizing enzyme of irinotecan, are important determinants of individual variations in susceptibility to toxicity.[5] Irinotecan is a prodrug that is metabolized by carboxylesterase to its principal active metabolite, SN-38. SN-38 is subsequently conjugated mainly by UGT1A1 to a more polar, inactive glucuronide (SN-38G). Severe toxicity is attributed, at least in part, to increased exposure to SN-38 caused by decreased UGT1A1 activity due to genetic polymorphisms. Pharmacogenetic studies of irinotecan toxicity have therefore focused on genetic polymorphisms of the *UGT1A1* gene, especially *UGT1A1*28*, a variant sequence in the promoter region.[6,7] Multiple studies have significantly linked this variant to irinotecan-related toxicity.[8] The United States Food and Drug Administration has required that the package insert of irinotecan states that *UGT1A1*28* is associated with an increased risk of toxicity; to decrease this risk, genetic testing is encouraged. The package insert also recommends that the starting dose of irinotecan is reduced by at least one level for patients who are homozygous for *UGT1A1*28*; however, whether this reduction is sufficient remains unclear. Firm recommendations for patients who are heterozygous for *UGT1A1*28* have yet to be established. Such patients are likely to have an intermediate UGT1A1 activity leading to an increased risk of neutropenia, but clinical evidence supporting the use of lower doses of irinotecan is lacking.

The clinical consequences of *UGT1A1*6* and *UGT1A1*27*, single nucleotide polymorphisms in exon 1 of the *UGT1A1* gene, also remain poorly understood. These variants are found mainly in Asians.[9] Unlike *UGT1A1*28*, case-control studies of Japanese patients have failed to demonstrate a significant relationship between *UGT1A1*6* alone and severe irinotecan-related toxicity.[10] However, the study suggested that the presence of *UGT1A1*6* and *UGT1A1*27* in addition to *UGT1A1*28* might increase susceptibility to irinotecan-related toxicity considerably. To better understand the clinical significance of these variants, especially the more frequent variant of *UGT1A1*6*, we examined how the coexistence of *UGT1A1*6* or *UGT1A1*27* and *UGT1A1*28* alters the pharmacokinetics of irinotecan in Japanese patients.

Materials and Methods

Patients. Forty-six Japanese patients with cancer who received irinotecan monotherapy or various regimens of irinotecan-based chemotherapy from June 2003 through April 2006 were studied. Some patients with metastatic colorectal cancer were also enrolled in a phase I study of a regimen containing irinotecan, fluorouracil (FU), and leucovorin (LV) (FOLFIRI), carried out at Saitama Medical School (Saitama, Japan). Toxicity was assessed prospectively according to the common terminology criteria for adverse events, version 3.0 (http://ctep.cancer.gov/reporting/ctc_v30.html). Tumor response was not included as a study variable because of various primary diseases and prior therapies. All subjects gave informed consent in writing for their peripheral blood samples and medical information to be used in this study. This study was approved by the Institutional Review Board of Saitama Medical School.

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Treatments. Irinotecan as a monotherapy was given weekly at doses of 50, 75 or 100 mg/m^2 for the first 3 weeks of a 4-week cycle or every 2 weeks at a dose of 150 mg/m^2 until the onset of disease progression or intolerable toxicity. For combination chemotherapy, a 100-mg/m^2 dose of irinotecan was administered with bolus FU 500 mg/m^2 and LV 10 mg/m^2 (L isomer form) weekly for the first 4 weeks of a 6-week cycle (IFL). The FOLFIRI regimen, administered at 2-week intervals, comprised irinotecan starting at 150 or 180 mg/m^2 and LV 200 mg/m^2 administered over the course of 2 h, followed by FU 400 mg/m^2 as a bolus injection and FU 2400 mg/m^2 as a 46-h continuous infusion. Regimens containing irinotecan plus cisplatin (IP) were repeated every 4 weeks. Irinotecan was given at a dose of 50–70 mg/m^2, followed 2 h later by a 120-min infusion of cisplatin 80 mg/m^2 with adequate hydration on day 1. The same dose of irinotecan was given again on day 15. For all regimens, 50–180 mg/m^2 of irinotecan was dissolved in 250 mL of 5% dextrose solution and infused over the course of 90 min

Pharmacokinetic analysis. Blood samples for pharmacokinetic analysis were usually obtained during the first course of treatment with irinotecan. The samples were taken from the arm opposite the infusion site, at the beginning of irinotecan infusion and 0, 0.25, 0.5, 1, 2, 4, 8 and 24 h after the end of the infusion. The blood samples were centrifuged immediately, and the plasma was stored at −80°C until analysis. Total (lactone and carboxylate) plasma concentrations of irinotecan, SN-38 and SN-38G were analyzed by a modified reverse-phase high-performance liquid chromatographic (HPLC) method.[11] A 150-μL plasma sample was mixed with 300 μL of methanol, 5% perchloric acid (50:50, v/v) and camptothecin, serving as an internal standard, in a vortex mixer. The mixture was centrifuged at 20 600 *g* for 10 min, and 200 μL of the supernatant was injected into an HPLC system (Hitachi model 7000 series; Hitachi, Tokyo, Japan), equipped with a TSK-gel ODS-120T analytical column (4.6 × 250 mm, 4 μm; TOSOH, Tokyo, Japan). HPLC was carried out at 40°C at a flow rate of 1.0 mL/min. Irinotecan, SN-38 and SN-38G were determined fluorometrically (excitation 355 nm; emission 515 nm). The total (lactone and carboxylate) concentrations of irinotecan, SN-38 and SN-38G in plasma were quantified. The mobile phase consisted of 75 mM ammonium acetate (pH 4.75) for solvent A, and acetonitrile for solvent B; a 20-min run was carried out with a linear gradient of 85–65% for solvent A.

The lower limit of quantification was 5 ng/mL (7.4 nM) for irinotecan and 0.5 ng/mL (1.2 nM and 0.88 nM, respectively) for SN-38 and SN-38G. The intra-assay and interassay coefficients of variation were less than 10% for irinotecan and the metabolites.

The area under the plasma time–concentration curve (AUC; μmol·h/L) from the beginning of the infusion of irinotecan to the time of obtaining the last blood sample was calculated by a linear trapezoidal rule. The ratio of the AUC of SN-38 to that of SN-38G ($AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$) was used as a surrogate for UGT1A1 enzyme activity *in vivo*.

Genotyping. Genomic DNA was extracted from peripheral blood, which had been stored at −80°C until analysis, with the use of a QIAamp Blood Kit (QIAGEN, Hilden, Germany).

To analyze exon 1, first-step PCR amplification of a 923-bp fragment containing exon 1 was carried out as reported previously.[10]

For analysis of *UGT1A1*6*, the second set of polymerase chain reaction (PCR) amplifications was carried out with nested primers designed to amplify a 235-bp segment. The mismatched forward primer and the reverse primer were 5′-CTAGCACCTGACGCCTCGTTGTACATCAGAGCC-3′ (+178 to +210; underlining indicates mismatched site) and 5′-CCATGAGCTCCTTGTTGTGC-3′ (+393 to +412), respectively. The forward primer was designed to introduce an *Msp*I (Takara Shuzo Co., Otsu, Japan) restriction site into the wild-type sequence from +209 to +212. A 1000-fold diluted product of the first PCR was subjected to nested PCR in a volume of 50 μL containing 0.2 mM of each deoxynucleoside triphosphate, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.5 μM of each primer and 1.3 units of *Taq* polymerase (AmpliTaq Gold; Perkin-Elmer, Foster City, CA, USA). The PCR conditions were 95°C for 15 min followed by 25 cycles of 94°C for 30 s, 55°C for 40 s and 72°C for 40 s. A 1-μL PCR product was digested with 4 units of *Msp*I for 1 h at 37°C. DNA from the wild-type sequence was digested into 203- and 32-bp fragments, whereas DNA from *UGT1A1*6* gave an undigested 235-bp fragment.

For the sequence of *UGT1A1*27*, another set of the second PCR amplifications was carried out using hemi-nested primers 5′-AGTACCTGTCTCTGCCCAC-3′ (+485 to +503) and 5′-GTCCCACTCCAATACACAC-3′ (+865 to +867 and intron 1), designed to amplify a 399-bp segment. Two *Bsr*I (New England Biolabs, Ipswich, MA, USA) restriction sites exist in *UGT1A1*27* (+552 to +556 and +684 to +688), but only one site (+552 to +556) exists in wild type. The method of PCR amplification was identical to that for the *Msp*I restriction fragment length polymorphism, as described above. Digestion of the PCR products with 2.5 units of *Bsr*I for 1 h at 65°C gave 199-, 132- and 68-bp fragments from *UGT1A1*27*, or 331- and 68-bp fragments from wild type.

*UGT1A1*28* was distinguished from wild type by direct sequencing (−147 to +106) of the 253–255 bp produced by PCR.

A set of PCR amplifications of a 1030-bp fragment containing *UGT1A1*28* and *UGT1A1*6* was used to determine the location of these variant sequences on the respective alleles. The reaction mixture was similar to that described previously,[7] except that the concentration of $MgCl_2$ was 2.0 mM, and the amount of *Taq* polymerase was 1.3 units. The PCR primers were 5′-AAGTGAACTCCCTGCTACCTT-3′ (−147 to −127) and 5′-GTCCCACTCCAATACACAC-3′ (+865 to +867 and intron 1). The PCR conditions were 95°C for 15 min, followed by 30 cycles of 94°C for 30 s, 58°C for 40 s and 72°C for 40 s. The PCR fragments obtained were subcloned into pT7 Blue T-Vector (Novagen, Darmstadt, Germany), and sequence analysis was used to examine the gene arrangement of the variants on homologous chromosomes.

Statistical analysis. The statistical significance of differences in the $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ was assessed using the Mann–Whitney *U* test. This and other statistical analyses were carried out with SPSS for Windows, version 12.0 J (SPSS Japan, Tokyo Japan). Differences were considered statistically significant when the two-tailed *P*-value was less than 0.05.

Results

A total of 46 patients were studied (Table 1). Performance status was generally good; most patients had gastrointestinal tract cancers. Fourteen patients received IP, 20 FOLFIRI, three IFL and nine irinotecan monotherapy. Two of the 14 patients treated with IP, which is one of the experimental arms of an ongoing randomized phase III trial against unresectable gastric cancer in Japan (JCOG9912), were enrolled into JCOG9912, and the other 12 patients treated with IP also fulfilled the recruitment criteria of JCOG9912 except for having no prior chemotherapy.

Genomic DNA from all patients was genotyped to examine *UGT1A1* polymorphisms. No patient was homozygous for *UGT1A1*28* or had *UGT1A1*27* (Table 2). Two patients were heterozygous for *UGT1A1*28*. Two patients were homozygous and 15 were heterozygous for *UGT1A1*6*, all of whom were wild type with respect to *UGT1A1*28*. Two patients were simultaneously heterozygous for *UGT1A1*6* and *UGT1A1*28*. Sequencing of the subcloned products obtained from the two

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Table 1. Patient characteristics ($n = 46$)

Characteristic	n
Median age (years)	62 (range: 42–85)
Sex	
Female	20
Male	26
Performance status	
0	28
1	16
2	2
Primary organ	
Gastric	14
Colorectal	25
Other	7
Previous treatments	
Surgery	31
Radiotherapy	3
Systemic chemotherapy	38
1 regimen	30
2 regimens	8
None	6

Table 2. UGT1A1 genetic profiles and plasma concentration–time curve (AUC) ratios

*UGT1A1*6*	*UGT1A1*28*	$n^{\dagger}$	$AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$
–/–	–/–	24	0.43 (0.08–1.39)[‡]
+/–	–/–	15	0.61 (0.26–1.42)[‡]
+/+	–/–	2	1.40, 1.10
–/–	+/–	2	0.48, 0.44
+/–	–/+	2	1.04, 2.16

[†]Total $n = 45$. One patient was excluded because of an incomplete set of blood samples; [‡]median (range).

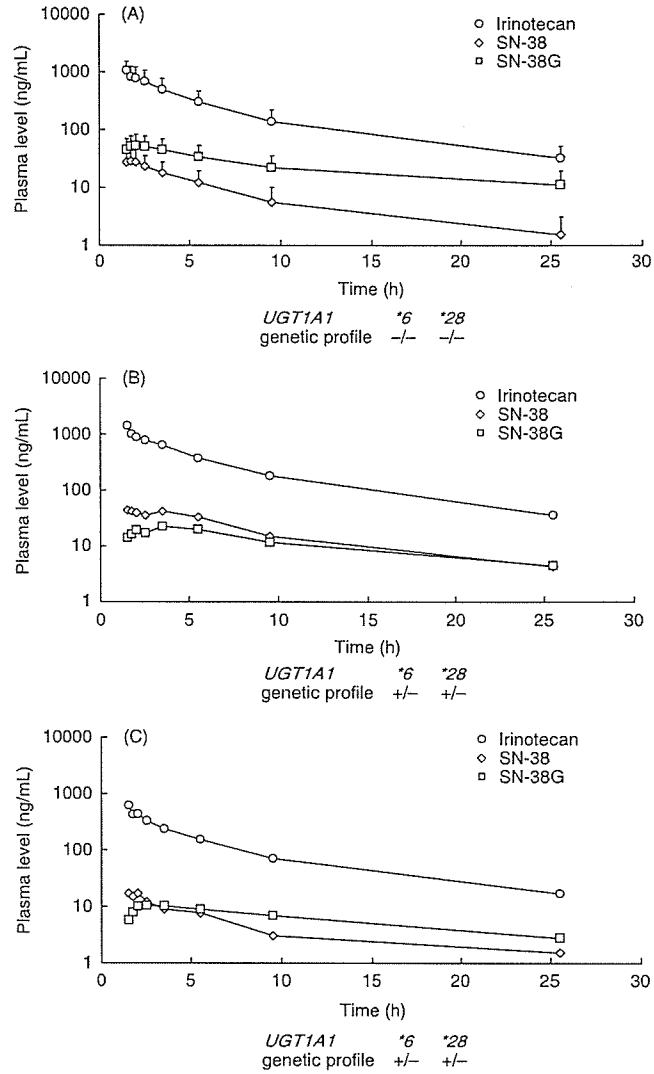

Fig. 1. Pharmacokinetic profiles of patients who received irinotecan. (A) The profile of 24 patients without *UGT1A1*28*, *UGT1A1*6* and *UGT1A1*27* showed that SN-38 glucuronide (SN-38G) levels were higher than the SN-38 levels reported in previous studies. Each data point indicates mean ± SD. (B) The patient with colon cancer received irinotecan at a dose of 150 mg/m² in FOLFIRI. (C) The patient with gastric cancer received irinotecan at a dose of 70 mg/m² in IP. SN-38G levels were lower than SN-38 levels in these two patients who were heterozygous for *UGT1A1*6* and *UGT1A1*28*.

patients who were heterozygous for both *UGT1A1*6* and *UGT1A1*28* revealed that the variants existed on different chromosomes (*UGT1A1*6/UGT1A1*28* diplotype), which was consistent with the results of Saeki *et al.*[11] The other 25 patients had none of the variants studied.

Complete sets of blood samples were obtained in 45 of the 46 patients. In the remaining one patient who received FOLFIRI, a blood sample could not be obtained for technical reasons at the end of the infusion. Pharmacokinetic profiling showed that SN-38G levels were higher than SN-38 levels; $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ was therefore usually under 1 (Table 2; Fig. 1A). There was no apparent relationship between $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ and the dose of irinotecan (data not shown). In the patient who was excluded from AUC analysis because of the missing blood sample, SN-38G levels were higher than SN-38 levels at all other time points. This patient had none of the variants studied.

In the two patients who had the heterozygous *UGT1A1*6/UGT1A1*28* diplotype, SN-38G levels were lower than or approximated SN-38 levels. Consequently, their $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ ratios were higher than those of the patients who had no variants or who were heterozygous for either *UGT1A1*6* or *UGT1A1*28* alone (Table 2; Figs 1B,C, 2). One of these patients who received the FOLFIRI regimen at an irinotecan dose of 150 mg/m² was studied again, after obtaining additional informed consent, to confirm reproducibility of the pharmacokinetic profile. The dose of irinotecan was reduced from 150 mg/m² in the first course to 100 mg/m² in the second course because of grade 4 neutropenia during the first course. The patient had grade 2 neutropenia in the second course. The pharmacokinetic profiles were similar for the first and second courses of treatment; the $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ was 2.16 in the first course and 1.56 in the second. The other patient who had the heterozygous *UGT1A1*6/UGT1A1*28* diplotype received IP safely, with no apparent toxicity.

Two patients were homozygous for *UGT1A1*6*. Their $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ ratios were relatively higher than those of the other patients (Table 2; Fig. 2). One received irinotecan at a dose of 180 mg/m² in the FOLFIRI regimen and had grade 2 neutropenia in the first course of treatment. Pharmacokinetic studies were repeated in this patient with the same dose of irinotecan after obtaining additional informed consent. The patient had grade 3 neutropenia in the second course. Similar pharmacokinetic profiles were obtained; the $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ ratio was 1.40

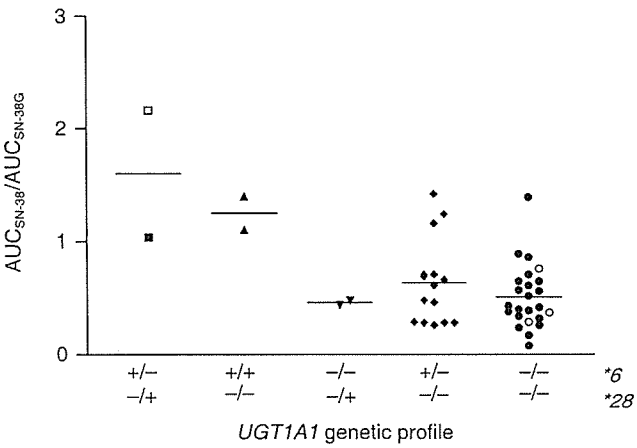

Fig. 2. Ratio of the area under the plasma concentration–time curve (AUC) for SN-38 to the AUC for SN-38 glucuronide (SN-38G), used as a surrogate for UGT1A1 activity (AUC_{SN-38}/AUC_{SN-38G}). Results are shown according to *UGT1A1* genetic profile for 45 Japanese patients who received various regimens of irinotecan chemotherapy. Open symbols in the figure represent the patients suffering from grade 4 neutropenia. Lines indicate median values. One patient was excluded because of an incomplete set of blood samples.

in the first course and 2.73 in the second. The other patient received irinotecan monotherapy at a dose of 75 mg/m^2 and had grade 3 nausea and grade 1 neutropenia. AUC_{SN-38}/AUC_{SN-38G} in this patient was 1.10. The AUC_{SN-38}/AUC_{SN-38G} ratios in the four patients who were heterozygous for *UGT1A1*28/UGT1A1*6* or homozygous for *UGT1A1*6* were significantly higher than those in the other 41 patients ($P = 0.0039$).

Among the 45 patients, four patients suffered from grade 4 neutropenia. Two of them received FOLFIRI regimen. One of the patients with *UGT1A1*28/UGT1A1*6* received a FOLFIRI regimen including irinotecan 150 mg/m^2, and the absolute AUC for SN-38 was the highest (1.06 μmol·h/L) among all patients who received FOLFIRI (median 0.56 μmol·h/L; range 0.49–1.06 μmol·h/L; irinotecan dose 150 or 180 mg/m^2; $n = 19$). One who had wild-type *UGT1A1* alleles received FOLFIRI with irinotecan 180 mg/m^2, and the absolute AUC for SN-38 was not high (0.66 μmol·h/L). Another two who harbored wild-type *UGT1A1* alleles received IP regimens with irinotecan 60 or 70 mg/m^2. The AUCs for SN-38 were, respectively, 0.39 μmol·h/L and 0.26 μmol·h/L, similar to the values in the other patients given IP (median 0.30 μmol·h/L; range 0.20–0.68 μmol·h/L; $n = 12$). No patient had grade 3 or higher diarrhea in this study.

Discussion

The present study demonstrated that patients who were heterozygous for both *UGT1A1*6* and *UGT1A1*28* (*UGT1A1*6/UGT1A1*28* diplotype) had markedly lower SN-38 glucuronidation activity than those who were heterozygous for either of these variants, let alone patients who had neither variant. Previous pharmacokinetic studies of irinotecan have reported that *UGT1A1*6* decreases SN-38 glucuronidation, despite the effects being somewhat lower than *UGT1A1*28* does.[12,13] Heterozygous *UGT1A1*28* status is considered to result in UGT activity intermediate between that of wild type and that of homozygous *UGT1A1*28*. Thus, the presence of both *UGT1A1*6* and *UGT1A1*28*, even when heterozygous, apparently lowers SN-38 glucuronidation activity additively, resulting in a phenotypic effect similar to that associated with homozygous *UGT1A1*28*. The mechanisms responsible for this add-on effect are unclear. *UGT1A1*6* and *UGT1A1*28* decrease UGT1A1 activity via different mechanisms; the former variant lessens protein function directly, whereas the latter reduces transcriptional activity of the promoter. Genetic effects on UGT activity compensate for each other when these variants exist separately. In contrast, compensation might be hindered by the coexistence of *UGT1A1*6* and *UGT1A1*28*, resulting in remarkably lower UGT activity.

Our results suggest that patients who have the *UGT1A1*6/UGT1A1*28* diplotype are at elevated risk for severe irinotecan-related toxicity due to increased exposure to SN-38. This notion is supported by the fact that one of the four patients with severe toxicity had the *UGT1A1*6/UGT1A1*28* diplotype. The AUC for SN-38 in this patient was higher than that of any other patients who received the same FOLFIRI regimen. We therefore believe that patients who have the *UGT1A1*6/UGT1A1*28* diplotype should be treated carefully, similar to those who are homozygous for *UGT1A1*28*. Furthermore, 2.5–4.6% of the Japanese population is estimated to have both *UGT1A1*6* and *UGT1A1*28*,[13–15] which is similar to, or slightly higher than, the percentage homozygous for *UGT1A1*28*. These findings suggest that pharmacogenetic testing for irinotecan toxicity should include not only *UGT1A1*28*, but also the coding region variant *UGT1A1*6*, at least in Japanese patients.

Although the patients who were homozygous for *UGT1A1*6* had lower SN-38 glucuronidation activity than the others in this study, *UGT1A1*6* alone appears to have only a limited effect on irinotecan-related toxicity. Indeed, *UGT1A1*6* was not associated with severe toxicity, and even patients who were homozygous for *UGT1A1*6* could be treated safely in a previous study.[10] Thus, patients who are heterozygous or even homozygous for *UGT1A1*6* alone can most likely tolerate conventional starting doses of irinotecan, unless they have other conditions associated with an increased risk of toxicity. In *in vitro* expression studies, however, homozygosity and heterozygosity for *UGT1A1*6* were associated with approximately 30 and 60% reductions in UGT activity, respectively.[16] Otherwise, *UGT1A1*6* could be partially linked to other genetic polymorphisms that decrease overall UGT activity. We have recently identified genetic linkages of *UGT1A7* and *UGT1A9* polymorphisms to *UGT1A1*6*, including those related to lower catalytic or transcriptional activities of UGT enzymes (K. Fujita *et al.*, unpublished data, 2006). In addition, a previous pharmacokinetic study showed that the patients who had *UGT1A1*6* alone showed slightly but not significantly decreased SN-38 glucuronidation activity,[13] consistent with the results of our study. As for hyperbilirubinemia, *UGT1A1*6* is a significant contributory factor to unconjugated hyperbilirubinemia, including Gilbert's syndrome, especially among Japanese neonates.[14,15] These findings suggest that patients who have *UGT1A1*6*, especially when homozygous, are at increased risk for irinotecan-related toxicity. Although definitive evidence of this risk is lacking, we believe that toxicity should be monitored rigorously during irinotecan chemotherapy in patients who have *UGT1A1*6* alone.

The genetic basis for irinotecan-related toxicity apparently differs among distinct ethnic populations. That is to say, the same genetic variants affect toxicity differently. *UGT1A1* genetic polymorphisms differ considerably among genetically distinct populations; the allele frequency of *UGT1A1*28* is several times higher in whites (0.3–0.4) than in Asians (around 0.15).[17,18] *UGT1A1*6* and *UGT1A1*27* have been identified only in Asians (0.11–0.23 for *UGT1A1*6* and 0.01–0.03 for *UGT1A1*27*).[6,9,12,14] As for hyperbilirubinemia, bilirubin levels are generally higher in Asians than in whites, at least among infants, suggesting that the genetic basis for hyperbilirubinemia differs between these two ethnic groups.[19–21] The neonatal hyperbilirubinemia found in Japanese is significantly related to *UGT1A1*6*, not *UGT1A1*28*,[14,15] whereas that occurring in whites during the first 2 days of life is associated with homozygosity

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for *UGT1A1*28*.[22] In the present study, the total bilirubin levels observed in the patients who were homozygous for *UGT1A1*6* and heterozygous for both of *UGT1A1*6* and *UGT1A1*28* were not significantly higher than those observed in the other patients. The complex genetic basis for UGT1A1 activity makes it difficult to compare irinotecan-related toxicity among different ethnic groups. In addition, other factors potentially related to irinotecan-associated toxicity include patients' age, organ functions, prior treatments, dosing schedule and concurrently administered drugs. However, because genotype-based individualized chemotherapy with irinotecan is now practiced widely, studies comparing irinotecan toxicity among the different ethnic populations appear to be warranted.

In conclusion, our study showed that the coexistence of two *UGT1A1* variants, the promoter variant *UGT1A1*28* and the coding region variant *UGT1A1*6*, remarkably altered the disposition of irinotecan, potentially increasing susceptibility to toxicity, even when these variants were heterozygous. Patients who had the *UGT1A1*6/UGT1A1*28* diplotype should therefore be treated similarly to those who are homozygous for *UGT1A1*28*. Genotyping for *UGT1A1*6* in addition to *UGT1A1*28* is necessary to predict the risk of irinotecan-related toxicity, at least in Asian patients. Genomic information is hereafter expected to play an increasingly important role in optimizing the use of irinotecan therapy.

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Late toxicity in complete response cases after definitive chemoradiotherapy for esophageal squamous cell carcinoma

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Background. We retrospectively investigated long-term toxicity after concurrent chemoradiotherapy (CRT) for patients with esophageal squamous cell carcinoma (ESCC). ***Methods.*** Concurrent chemoradiotherapy was performed in 110 patients with T1 to T4 disease containing M1 lymph node (LYM) disease. Chemotherapy consisted of protracted infusion of 5-fluorouracil 400 mg/m^2 per 24 h on days 1 to 5 and 8 to 12, combined with 2-h infusion of cisplatin 40 mg/m^2 on days 1 and 8. Radiation treatment of the mediastinum at a dose of 30 Gy in 15 fractions was administered concomitantly with chemotherapy. A course schedule with a 3-week treatment and a 2-week break was applied twice, with a total radiation dose of 60 Gy. For the assessment of toxicity, the Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer late radiation morbidity scoring schema was adopted. ***Results.*** A total of 81 patients were recruited in patients with stage I to IVA. Of 34 patients with complete response, 1 patient died as a result of acute myocardial infarction. Grade 2, 3, and 4 late toxicities occurred with the following incidences: pericarditis in 3 patients, 1 patient, and 2 patients, respectively; heart failure in 0, 0, and 3 patients; pleural effusion in 2, 3, and 0 patients; and radiation pneumonitis in 0, 0, and 1 patient, respectively. ***Conclusions.*** Definitive chemoradiotherapy for ESCC is effective with substantial toxicities. Further investigation is warranted to minimize the normal tissue toxicities.

Key words: esophageal carcinoma, chemoradiotherapy, late toxicity, prognosis

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Introduction

In Western countries, the number of patients with esophageal adenocarcinoma has been increasing, whereas most patients in Japan have squamous cell carcinoma of the esophagus. Previous studies indicated that 95% of esophageal cancers in Japanese patients are squamous cell carcinomas.[1] In recent years, most patients are still diagnosed with advanced-stage ESCC, although the number of patients found with early-stage ESCC has been increasing. The standard therapy in Japan for patients with resectable disease has been surgery. The 5-year survival rate for T1, T2, and T3 disease was 52%, 37%, and 28%, respectively.[2]

On the other hand, the effects of chemotherapy combined with radiotherapy on esophageal carcinoma have been investigated since the 1980s. Several investigators have reported successful results with these modalities, either with or without surgery, against locoregional carcinoma.[3–8] The combination of 5-fluorouracil (5-FU) and cisplatin (CDDP) has become a standard regimen, not only because of the clinical outcome, but also because of the synergism between the two agents and their radiosensitizing effects.[9–11] Recently published results on chemoradiotherapy (CRT) indicated that it offers various advantages for the treatment of carcinoma of the esophagus.[7,12] In a prospective randomized trial by the Radiation Therapy Oncology Group, which compared chemoradiotherapy with radiotherapy alone, the combined-modality arm demonstrated a significant improvement of survival;[13] with a 5-year survival rate of 27%, compared with 0% for radiotherapy alone.[14] With regard to the indications for CRT as a curative treatment for patients with locally advanced diseases, our multicenter study suggested that concurrent CRT was potentially curative even in cases with unresectable carcinoma of the esophagus (i.e., T4 and/or M1 LYM disease).[15,16]

Esophageal cancer deaths often occur in non-CR cases or in recurrent cases. However, recent data indi-

cate that the risk of early death from esophageal carcinoma is not quite as daunting for patients who achieve complete response (CR) after CRT. We have already reported that concurrent CRT was effective for inoperable patients. Therefore, a significant proportion of CR patients may have a sufficiently long survival time to allow for the adequate assessment of treatment-related late toxicity. We retrospectively investigated the long-term toxicity after definitive CRT for patients with squamous cell carcinoma of the thoracic esophagus.

Methods

Patient population

From May 1996 through March 2002, 110 consecutive patients were diagnosed at Showa University School of Medicine as having esophageal carcinoma. Patients were recruited from our database on the basis of the following criteria: age ≤ 75 years, performance status (Eastern Cooperative Oncology group) 0 to 2, clinical stage I to IVA (International Union Against Cancer tumor-node-metastasis system, 1997), adequate organ function, and no other site of carcinoma except for early stage. None of the patients had surgery or chemotherapy for previous diseases. In agreement with that observation, none of the patients enrolled in this study had esophageal adenocarcinoma; thus, all patients had squamous cell carcinoma of the esophagus.

Eligibility criteria

Patients who were eligible for this trial had previously untreated, histologically confirmed squamous cell carcinoma of the thoracic esophagus. The tumors had to show evidence of T1–T4 disease, containing M1 LYM disease, based on the staging criteria of the UICC. The prestudy clinical evaluation included air-contrast barium esophagography, esophagoscopy, neck computed tomography (CT), chest CT, abdominal CT, endoscopic ultrasonography (EUS), bronchoscopy, and bone scan. However, EUS was optional because the endoscope could not be passed through stenotic lesions in most cases (68%). Bronchoscopy was performed in some cases when tracheobronchial involvement was suspected. Because the prognosis of patients with T4 disease differed significantly from that of patients with T3 disease,[16] we defined T3 and T4 disease in clinical staging. Adjacent organs were considered to be involved (T4 disease) if the tumors extended into the esophageal lumen, or caused deformity of the tracheobronchial tree, or if the tumors appeared to be attached to the organs at >90° angle to the thoracic aorta as observed on the CT scan.[17,18] T3 or lesser extent of the disease was determined by EUS. In those patients who could not undergo this procedure, T3 was defined based on the lack of any associated abnormal bronchoscopic findings; i.e., no deformity of the airway and tracheobronchial tree on the CT scan. Furthermore, we modified the criteria described previously for the definition of T3 disease[17] to include tumors attached to the organs at ≤90° angle to the thoracic aorta as observed on the CT scan. The patients were considered to have lymph node metastasis if the tumor was ≥1 cm in diameter.[19] Radiologic evaluations for staging were reviewed by radiologists and medical oncologists at Showa University School of Medicine, as was reported in previous studies.[17–19] However, although the UICC staging criteria were adopted in these previous studies, we used a nonstandard staging technique in this study, especially when evaluating the depth of tumor infiltration.

The following criteria were used for enrollment for chemoradiotherapy: (1) an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 2 or less, (2) satisfactory hematological function (leukocyte count ≥3000/mm^3 and platelet count ≥100 000/mm^3), (3) satisfactory hepatic function [aspartate aminotransferase (AST) or alanine transferase (ALT) levels within three times the normal upper limit and a serum bilirubin level <2.0 mg/dl), (4) good renal function (creatinine level ≤1.5 mg/dl and creatinine clearance ≥50 ml/min), (5) satisfactory pulmonary function ($PaO_2 \geq 70$ mm Hg), (6) normal electrocardiogram, and (7) life expectancy ≥8 weeks. Patients with serious complications, such as history of ischemic heart disease, pulmonary fibrosis, or active carcinoma at another site, were excluded from the study. After explaining the true disease status and predicted complications of the treatment, including the possibility of treatment-related death, each patient gave informed consent for the study. The study protocol was approved by the Human Ethics Review Committee of Showa University School of Medicine.

Treatment schedule

Chemotherapy consisted of protracted infusion of 5-FU at a dose of 400 mg/m^2 per day on days 1–5 and 8–12, combined with 2-h infusion of CDDP at 40 mg/m^2 on days 1 and 8 (Fig. 1). A 10-MV radiation treatment was administered for 3 weeks (5 days/week) at 2 Gy/day, concomitantly with chemotherapy. The targeted area for carcinoma of the upper and middle third of the esophagus included the primary tumors with a 3-cm margin craniocaudally and any metastatic nodes with 1- to 1.5-cm margin, in the supraclavicular fossa and mediastinum. For carcinoma of the lower third of the esophagus, the field was extended to include the

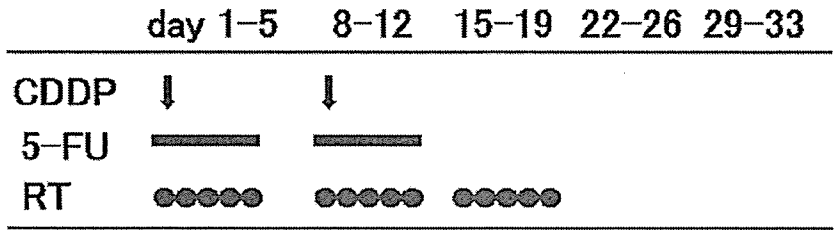

Fig. 1. The schema of treatment schedule for chemoradiotherapy. The treatment course included 3 weeks of radiotherapy followed by a 2-week break, and the course was repeated twice, with a total radiation dose of 60 Gy. *RT*, radiation treatment

perigastric nodes, while the supraclavicular fossa was excluded if the cervical nodes were found to be negative. The daily fractional dose of radiotherapy was 2 Gy administered 5 days a week. When the planned volume included both the supraclavicular fossa and upper abdominal nodes, the daily dose of 1.8 Gy was allowed. After a dose of 30 Gy, we allowed a 2-week treatment-free period. Radiotherapy was restarted on day 36, along with the same schedule of chemotherapy as described above. The treatment course included 3 weeks of radiotherapy followed by a 2-week break, and the course was repeated twice, with a total radiation dose of 60 Gy. The irradiation techniques were initially applied in anterior and posterior opposed fields. At 40 Gy, the radiation portals were reduced to shield the spinal cord and to encompass the primary tumor craniocaudally with a 2- to 3-cm margin, usually by using an oblique opposed field. Metastatic nodes were encompassed with a 1- to 1.5-cm margin. The total radiation dose to the spinal cord was kept at a maximum of 40 Gy. The homogeneity of the dose within the planning volume was within ±10% of the prescribed dose.

Patients who were evaluated for an objective response to this treatment received additional chemotherapy consisting of continuous infusion of 5-FU at a dose of 800 mg/m^2 on days 1–5 and CDDP at a dose of 80 mg/m^2 on day 1. This treatment schedule of 1-week treatment followed by a 3-week break was only repeated once in some patients, and no further treatment was applied if no disease progression was observed. When a single course consisted of treatment followed by >5-week break, we defined the latter as interruption. All patients receiving CRT were monitored by neck CT, chest CT, abdominal CT, endoscopy, and air-contrast esophagography every 4 weeks.

Evaluation of response and toxicity of chemoradiotherapy

For measurable lesions, response was assessed using the World Health Organization criteria. Briefly, a complete response (CR) was defined as the complete disappearance of all measurable and assessable disease for at least 4 weeks. A partial response (PR) was defined as more than 50% reduction in the sum of the products of the longest perpendicular diameter of measurable disease for a period of at least 4 weeks. Stable disease (SD) was defined as the failure to observe CR, PR, or progressive disease for at least 4 weeks. Progressive disease (PD) was defined as >25% increase in the sum of the products of the longest perpendicular diameter of measurable disease or the appearance of new lesions.

For primary tumors, CR was defined as when all visible tumors, including ulceration, disappeared for at least 4 weeks, confirmed by normal endoscopic biopsy specimens. Uncertain CR was defined as the persistence of small nodes (≤1 cm) with no evidence of progression for ≥3 months after completion of treatment, and patients with uncertain CR were included in the analysis of those with CR. The response was evaluated by esophagography, esophagoscopy, and neck, chest, and abdominal CT scans during each course.

Toxicity was evaluated using the criteria defined by the National Cancer Institute Common Toxicity Criteria (NCI-CTC, version 2.0). Toxicity was assessed on a weekly basis during chemoradiotherapy and then biweekly during the subsequent chemotherapy. Late toxicity assessment for cardiac ischemia, pericardial effusion, heart failure, and pleural effusion was performed according to the NCI-CTC. Radiation Therapy Oncology Group (RTOG)/European Organization for Research and Treatment of Cancer (EORTC) late radiation morbidity scoring schema was used for assessment of radiation pneumonitis. Late toxicity was defined as that occurring more than 90 days after the treatment initiation.

Statistical analysis

Follow-up evaluations after CRT were performed every 3 months for the first 2 years and every 6 months thereafter by endoscopy and CT scan. Differences between the two groups were calculated by the chi-square test. Survival was calculated from the data at the initiation of treatment by the actuarial Kaplan–Meier method.[20] Survival differences between the two groups were assessed by the log-rank test. *P* values less than 0.05 were considered significant.