GraphPad Prism version 5 software (GraphPad Software), using the equations for competitive inhibition, noncompetitive inhibition, or mixed inhibition [7]. The type of inhibition was determined from the enzyme inhibition models fitted to the data. Goodness of fit to the inhibition models was estimated from the F statistics, R^2 values, parameter standard error estimates, and 95% confidence intervals. Kinetic constants (K_m, V_{max}, and K_i) were reported as the means ± standard error.

Results

Inhibition of UGT1A1 by nilotinib

The substrate concentration versus SN-38 glucuronidation plots in the presence or absence of nilotinib is shown in Fig. 1a. Apparent K_m and V_{max} were calculated to be 6.80 ± 0.32 μM and 110 ± 1.8 pmol/min/mg prot., respectively. Nilotinib inhibited the SN-38 glucuronidation by UGT1A1 in a noncompetitive manner (Fig. 1b). The Eadie-Hofstee plots of SN-38 glucuronidation showed linearity with an R^2 value of 0.96, indicating that SN-38 glucuronidation was catalyzed by a single enzyme of UGT1A1 expressed in the HLM (Fig. 1c). Nonlinear regression analysis also revealed noncompetitive inhibition with R^2 of 0.99 (Fig. 1a). The K_i value was estimated to be 0.286 ± 0.0094 μM (Fig. 1a).

Apparent inhibition kinetics of nilotinib toward UGT1A1-mediated SN-38 was further examined with the recombinant human UGT1A1. Apparent K_m and V_{max} were calculated to be 4.18 ± 0.19 μM and 85.6 ± 1.3 pmol/min/mg prot., respectively (Fig. 2a). The K_m value obtained with the recombinant UGT1A1 was almost similar to that with HLM. Nilotinib inhibited the SN-38 glucuronidation by UGT1A1 in a noncompetitive manner (Fig. 2b). Nonlinear regression analysis also demonstrated noncompetitive inhibition with R^2 of 0.98 (Fig. 2a). The K_i value was estimated to be 0.079 ± 0.0029 μM (Fig. 2a).

Estimation of nilotinib-induced drug–drug interaction through UGT1A1

Inhibition of UGT1A1 activity by nilotinib may cause drug–drug interactions involving UGT1A1-catalyzed metabolism. The increase in the area under the plasma concentration–time curve (AUC) of other drugs by nilotinib can be estimated by using the K_i values obtained in this study using the method described by Ito et al. [8]. The average systemic plasma concentration of nilotinib after repeated oral administration ($[I]_{av}$) and the maximum unbound hepatic input concentration of nilotinib ($[I]_{in,u}$) are calculated as follows:

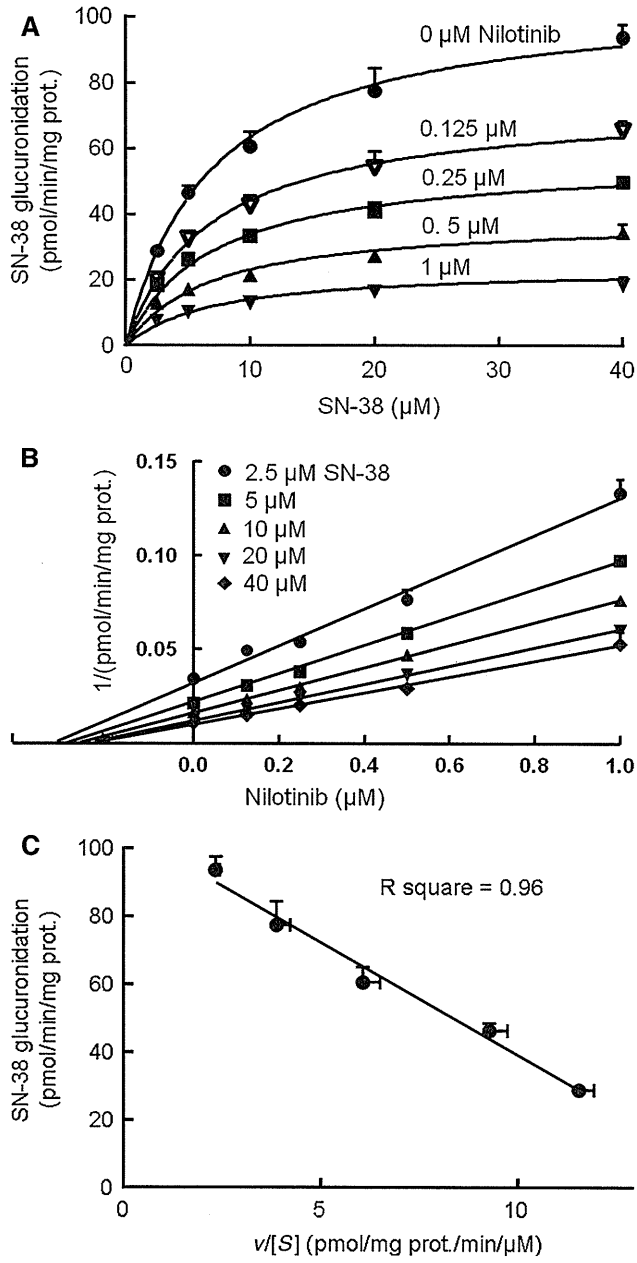

Fig. 1 Inhibition kinetics of nilotinib on SN-38 glucuronidation by UGT1A1 examined with human liver microsomes. **a** SN-38 concentration versus SN-38 glucuronidation plots. **b** Dixon plots. **c** Eadie-Hofstee plots of SN-38 glucuronidation in the absence of nilotinib. *v*, *Velocity* of glucuronidation; [*S*], SN-38 concentration. Each point shows the mean of three independent experiments with standard deviation

$$[I]_{av} = D/\tau/(CL/F) \quad (1)$$

$$[I]_{in,u} = fu\ ([I]_{av} + ka\ Fa D/Qh) \quad (2)$$

where D, τ, and CL/F are the dose, the dose interval and oral clearance of nilotinib, fu is the plasma unbound fraction, ka is the absorption rate constant, Fa is the extent of absorption, and Qh is the hepatic blood flow rate. The package inserts of nilotinib in the United States and Japan

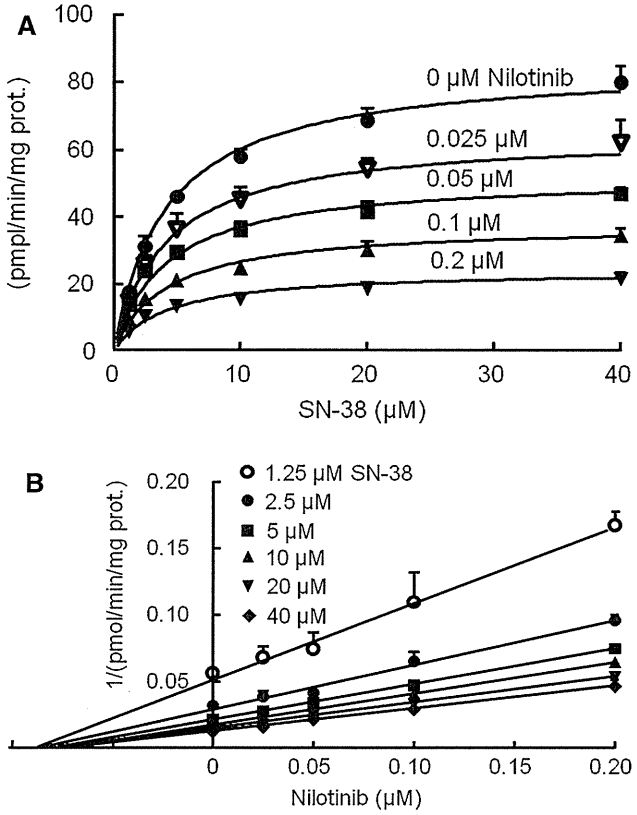

Fig. 2 Inhibition kinetics of nilotinib on SN-38 glucuronidation by recombinant human UGT1A1 **a** SN-38 concentration versus glucuronidation of SN-38. **b** Dixon plots. Each point represents the mean of three independent analyses with standard deviation

state that D and τ are 400 mg (0.685 mmol) and 12 h, respectively. CL/F obtained in fasting, Fa, and fu were reported to be 32.8 L/h, 0.3, and 0.02, respectively [9]. Since the ka value for nilotinib has not yet been reported, the value was assumed to be 0.1 min^{-1}, as proposed by Ito et al. [8]. The Qh was assumed to be 1,610 mL/min [8]. The AUC ratio in the presence or absence of nilotinib was used to estimate the potency of nilotinib to increase the AUC of a simultaneously administered drug that serves as a substrate of UGT1A1.

$$\text{AUC ratio} = 1 + [\text{I}]_{\text{in,u}}/K_i \quad (3)$$

The increase in the AUC was calculated to be 2.0 and 4.7 by using the K_i values obtained with HLM and recombinant human UGT1A1, respectively, even though we used the plasma unbound nilotinib concentration to reduce the possibility of false-positive estimates, suggesting the high risk of drug–drug interactions involving nilotinib [8].

Discussion

Our in vitro study showed that nilotinib was a potent noncompetitive inhibitor of human UGT1A1. The K_i values of UGT1A1 inhibition by nilotinib obtained with HLM and recombinant human UGT1A1 were 0.286 and 0.079 μM, respectively.

Singer et al. [5] suggested that the combined impact of the inhibition of UGT1A1 activity by nilotinib and genetic polymorphisms of *UGT1A1*, which were related to reduced expression or activity of UGT1A1, increased the incidence of hyperbilirubinemia. However, there has been no direct evidence on the inhibition of UGT1A1 by nilotinib [4, 5]. Our present results provide the first direct evidence for the in vitro inhibition of UGT1A1 activity by nilotinib and might support the mechanism of hyperbilirubinemia proposed by Singer et al. [5].

The increase in the AUC ratio of other drug to nilotinib was estimated to be higher than 2.0, suggesting the high risk of drug–drug interactions involving nilotinib [8]. Food intake has been shown to significantly affect the absorption of nilotinib [9]. Increased absorption of nilotinib was most pronounced after a high-fat meal, associated with an 82% increase in the AUC. Therefore, the AUC ratio may exceed 2.0, when nilotinib is taken after ingesting such foods. Caution should therefore be exercised when a drug serving as a substrate of UGT1A1 is administered with nilotinib.

The genetic polymorphisms in *UGT1A1* that cause lower expression of the protein (e.g., **28*) or lower catalytic activity of the enzyme (e.g., **6*) seen in patients with Gilbert's syndrome may further increase the AUC ratio as suggested by Singer et al. [5].

Interestingly, the FDA report [4] proposed that nilotinib competitively inhibited UGT1A1 activity, which does not agree with our findings. The reason for this difference is unclear because the FDA report did not include any experimental data.

Apparent K_m for SN-38 glucuronidation by UGT1A1 expressed in the HLM was concordant with the previous results [10]. V_{max} for the enzymatic reaction was about twice as high as that previously reported [10].

We compared the inhibition potency of SN-38 glucuronidation by nilotinib with several other small-molecule tyrosine kinase inhibitors. The lowest concentration that inhibited 50% of the maximal SN-38 glucuronidation by human UGT1A1 was obtained with nilotinib (0.146 μM), followed by sorafenib (0.446 μM), erlotinib (0.457 μM), lapatinib (1.47 μM), dasatinib (1.52 μM), gefitinib (3.50 μM), imatinib, and sunitinib (>10 μM). The results indicate that nilotinib is the most potent UGT1A1 inhibitor among small-molecule tyrosine kinase inhibitors tested.

We found that nilotinib is a potent noncompetitive inhibitor of UGT1A1. The result is applicable for the appropriate clinical management of the use of nilotinib with medicines predominantly metabolized by UGT1A1.

Acknowledgments We sincerely thank Drs. Yu, Sunakawa, Hiroo Ishida, Keishi Yamashita, Keiko Mizuno, Taro Yokoyama, Ken Shiozawa, Keisuke Miwa, Shigehira Saji, and Takashi Hirose for the valuable discussion on the present analyses. This study was supported in part by the Grant-in-Aid for Cancer Research 21S-8-1 from the Ministry of Health, Labour and Welfare of Japan, and in part by a grant-in-aid for "Support Project of Strategic Research Center in Private Universities" from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) to Saitama Medical University Research Center for Genomic Medicine.

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JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2011;41(2)204–209
doi:10.1093/jjco/hyq197
Advance Access Publication 21 October 2010

Phase I/II Study of FOLFIRI in Japanese Patients with Advanced Colorectal Cancer

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Received August 5, 2010; accepted September 20, 2010

Objective: This phase I/II study determined the recommended dose of FOLFIRI (irinotecan, infusional 5-fluorouracil and leucovorin) for Japanese patients with advanced colorectal cancer, and evaluated safety at the recommended dose in patients without the *UDP-glucuronosyltransferase 1A1*28* allele which caused reduced enzyme expression.
Methods: The phase I part assessed the maximum tolerated dose of FOLFIRI to determine the recommended doses of irinotecan and infusional 5-fluorouracil. The doses were escalated from 150 to 180 mg/m^2 (irinotecan) and 2000 to 2400 mg/m^2 (5-fluorouracil). *UDP-glucuronosyltransferase 1A1*6* and **28*, and pharmacokinetics of irinotecan were observationally examined. In the phase II part, patients without the *UDP-glucuronosyltransferase 1A1*28* allele received FOLFIRI at the recommended dose to evaluate safety.
Results: Among 15 patients in the phase I part, dose-limiting toxicity (diarrhea) occurred in one patient who received 150 mg/m^2 irinotecan and 2400 mg/m^2 infusional 5-fluorouracil. The respective recommended doses were 180 and 2400 mg/m^2 for irinotecan and infusional 5-fluorouracil, without reaching the maximum tolerated dose. Twenty-five patients received FOLFIRI at the recommended doses. Grade 3 or 4 neutropenia occurred in 44%, and Grade 3 diarrhea in 4%.
Conclusions: This phase I/II study demonstrates that the recommended doses of irinotecan and infusional 5-fluorouracil in FOLFIRI for Japanese patients with advanced colorectal cancer who do not possess the *UDP-glucuronosyltransferase 1A1*28* allele are 180 and 2400 mg/m^2, respectively. Toxicities occurring at the recommended doses are manageable in these patients.

Key words: FOLFIRI – recommended dose – Japanese – safety – UGT1A1 genotyping

INTRODUCTION

FOLFIRI, infusional 5-fluorouracil (5-FU) and *l*-leucovorin (*l*-LV) plus irinotecan, was developed in Europe and is now widely used as one of the standard treatment regimens for advanced colorectal cancer (CRC) (1,2). The doses of irinotecan and infusional 5-FU in the FOLFIRI regimen used in Western countries are 180 mg/m^2 and 2400–3000 mg/m^2, respectively, repeated every 2 weeks (1,2). In Japanese patients, however, the maximum tolerated doses (MTD) of irinotecan and infusional 5-FU in FOLFIRI remain uncertain. We routinely use irinotecan at a dose of 150 mg/m^2 in FOLFIRI. This dose has been approved for irinotecan monotherapy every 2 weeks by the Japanese Ministry of Health, Labour and Welfare.

Several lines of evidence have linked irinotecan toxicity to the *UGT1A1*28* allele. Patients homozygous for *UGT1A1*28* carry a significantly higher risk of severe irinotecan-related adverse events than those who do not possess this genotype (3,4) because *UGT1A1*28* decreases UGT1A1 protein expression and reduces the glucuronidation capacity for SN-38. In Asians, a specific mutation, *UGT1A1*6* (5), has been proved to reduce the catalytic activity of UGT1A1 (6,7). The *UGT1A1*28/*28*, **6/*6* and **6/*28* genotypes have been shown to be related to severe neutropenia in Asian populations (8–10).

We performed a dose-finding phase I study of irinotecan and continuous infusional 5-FU in the FOLFIRI regimen in Japanese patients with advanced CRC. We also observationally examined the *UGT1A1* genotyping and irinotecan pharmacokinetics in the phase I part to investigate the relation between them and FOLFIRI-related toxicity. Then, we evaluated the safety and efficacy of FOLFIRI at the recommended dose (RD) in patients without the *UGT1A1*28* allele. Here, we report the results of the first Japanese phase I and II study of FOLFIRI with *UGT1A1* genotyping.

PATIENTS AND METHODS

Eligibility

This study enrolled patients with histologically confirmed advanced CRC. Eligibility criteria included an age of $\leq$75 years; an Eastern Cooperative Oncology Group (ECOG) scale performance status of 0 or 1; no previous chemotherapy for at least 4 weeks; no previous irinotecan-based chemotherapy; adequate bone marrow (absolute neutrophil count $\geq$2000/μl, platelet count $\geq$100 000/μl), liver [serum total bilirubin $\leq$ upper limit of normal (ULN), serum aspartate aminotransferase and alanine aminotransferase $\leq$3.0 $\times$ ULN] and renal (serum creatinine $\leq$1.5 $\times$ ULN) functions; no severe medical conditions; no brain metastasis and no prior radiotherapy of the pelvis. The Institutional Review Board of Saitama Medical University approved the study protocol. Patients signed written informed consent for their peripheral blood samples and medical information to be used for research purposes.

Study Objectives

Phase I Part

The primary objective of the phase I part of this study was to assess the MTD and dose-limiting toxicity (DLT) of irinotecan and infusional 5-FU in FOLFIRI during the first course of treatment in patients with advanced CRC and thereby determine the RD. *UGT1A1*28* and **6* and the pharmacokinetics of irinotecan, the active metabolite of irinotecan SN-38 and the inactive metabolite SN-38 glucuronide (SN-38G) were observationally examined to evaluate the relations of *UGT1A1* genotype to irinotecan pharmacokinetics and irinotecan-induced adverse events. Pharmacogenetic and pharmacokinetic information were not reflected for patient enrollment and dose escalation in the phase I part.

Phase II Part

In the subsequent phase II part of the study, we excluded patients who had at least one *UGT1A1*28* allele, because we had considered that these patients were at higher risk in irinotecan-induced severe toxicities based on the report by Ando et al. (3). The primary objective of the phase II part was to evaluate the safety of FOLFIRI at the RD. The secondary objective was to assess response.

Treatment and Dose Escalation

FOLFIRI comprised a 2-h intravenous infusion of *l*-LV (200 mg/m^2) and a 90-min intravenous infusion of irinotecan (each level as described below) on day 1, followed by an intravenous bolus injection of 5-FU (400 mg/m^2) and a 46-h intravenous infusion of 5-FU (each level as described below); treatment was repeated every 2 weeks. Two sessions of treatment were counted as one course. The starting doses (Level 1) of irinotecan and infusional 5-FU were 150 and 2000 mg/m^2, respectively. The dose of infusional 5-FU was increased to 2400 mg/m^2 (Level 2). Then, the doses of irinotecan and infusional 5-FU were elevated to 180 and 2400 mg/m^2, respectively (Level 3). If patients did not tolerate Level 1, the dose of irinotecan was decreased to 120 mg/m^2 (Level 0).

DLT was defined as Grade 4 neutropenia lasting for more than 5 days, neutropenic fever (Grade 3 or 4 neutropenia with fever $\geq$38.5°C), Grade 4 thrombocytopenia, Grade 3 thrombocytopenia with hemorrhage or Grade 3 or higher non-hematologic toxicity during the first course of treatment. Three patients were initially enrolled at each dose level. If none of the first three patients had DLT, the dose was escalated, and three additional patients received the next dose level. If one of the three patients had DLT, then three additional patients were enrolled at the same dose level, and escalation to the next dose level was continued if only one of the six patients had DLT. If DLT occurred in more than one of the first three patients or more than one of six patients treated at any given dose level, dose escalation was stopped, and that level was defined as MTD. If DLT occurred at dose Level 1, then the dose level was decreased to Level 0. If DLT occurred at dose Level 0, the study was stopped. Patients who received Level 0 and had DLT were treated with FOLFIRI at an irinotecan dose of 100 mg/m^2 (Level $-$1) after resolution of toxicity as evaluated by the physician in charge. In principle, the RD was defined as the dose one level below the MTD, but toxic effects occurring in later courses were also considered. Six patients were enrolled at the RD.

After determining the RD in the phase I part of the study, additional patients received FOLFIRI at the RD in the phase II part.

Toxicity was assessed weekly during the first course and every 2 weeks during the second and subsequent courses according to the National Cancer Institute Common Toxicity Criteria version 2.0 (http://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcv20_4-30-992.pdf). Chemotherapy was delayed until recovery if the leukocyte count was $<3000/\mu l$, the platelet count was 100 000/μl or if clinically significant, persistent non-hematologic toxicity occurred. Tumor response was evaluated every two courses according to the standard World Health Organization response criteria (11). Treatment was continued until disease progression, unacceptable toxicity or the patient's refusal of further treatment.

UGT1A1 Genotyping

Genomic DNA was extracted from 200 μl of peripheral blood, which had been stored at −80°C until analysis, with the use of a QIAamp Blood Kit (QIAGEN GmbH, Hilden, Germany). The polymorphism *UGT1A1*6* was analyzed by the polymerase chain reaction-restriction fragment length polymorphism method as described elsewhere (12). *UGT1A1*28* was determined by the direct sequencing method as described by Fujita et al. (12).

Pharmacokinetic Analysis of Irinotecan and its Metabolites

Blood samples for pharmacokinetic analysis were obtained during the first treatment with FOLFIRI in the patients who agreed to have blood sampling for phamacokinetics. The samples were taken from the arm opposite the infusion site at the beginning of irinotecan infusion and at 0, 0.25, 0.5, 1, 2, 4, 8 and 24 h after the end of the 1.5 h infusion. The samples were immediately centrifuged, 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 reverse-phase high-performance liquid chromatography as described by Araki et al. (8). The lower limits of quantification were 5 ng/ml (7.4 nM) for irinotecan and 0.5 ng/ml (1.2 and 0.88 nM) for SN-38 and SN-38G. The intra-assay and inter-assay coefficients of variation for irinotecan and its metabolites were $<10\%$.

Pharmacokinetic Parameters

The plasma concentration–time data of irinotecan and its metabolites were analyzed by a standard non-compartmental method, using WinNonlin version 5.2 software (Pharsight Corporation, Mountain View, CA, USA). The area under the plasma concentration–time curve (AUC) for time zero to the last sampling was calculated with the linear trapezoidal rule (until the peak plasma concentration) and linear-log trapezoidal rule (until the last quantifiable concentration).

Statistical Design in Phase II Part

In the phase II part of this study, we set a response rate of 25% as the target activity level and chose 5% as the lowest response rate of interest. According to Simon's two-stage minimax design with an α level of 10 and 90% power (13), we planned to enroll at least 20 patients at the RD, with at least one response among 13 patients in the first step being required for this regimen to be considered worthy of further evaluation. The exact confidence interval for the response rate was calculated based on binomial distribution.

RESULTS

Patient Characteristics

Between March 2005 and April 2007, a total of 34 patients were enrolled into this phase I/II study, 15 in phase I and 19 in phase II. The baseline characteristics of the patients are shown in Table 1. The median age was 61 years (range, 35–75). Twenty-three (67%) patients had received at least one prior regimen of chemotherapy.

Table 1. Baseline characteristics of all patients enrolled in the present phase I/II study

Characteristics	Number of patients
Age (years)	61 (35–75)[a]
Gender	
Male	21
Female	13
ECOG performance status	
0	31
1	3
Primary tumor	
Colon	21
Rectum	13
Number of metastases	
1	20
2	10
3	4
Number of prior chemotherapy regimens	
0	11
1	21
≥2	2

ECOG, Eastern Cooperative Oncology Group.
[a]Median (range).

Table 2. Dose escalation and dose-limiting toxicity in patients treated with FOLFIRI

Dose level	Irinotecan (mg/m^2)	5-FU (mg/m^2)	Toxicities[a]	*UGT1A1*		AUC (μM h)			$AUC_{SN-38}/AUC^{c}_{SN-38G}$
				**28*	**6*	Irinotecan	SN-38	SN-38G	
1	150	2000		−/−	+/−	—	—	—	—
				−/−	−/−	—	—	—	—
				−/−	−/−	—	—	—	—
2	150	2400		−/−	+/−	—	—	—	—
				−/−	−/−	7.27	0.36	0.41	0.89
				−/−	+/+	—	—	—	—
			Diarrhea (Grade 3)[b]	−/−	−/−	—	—	—	—
			Leukopenia/neutropenia (Grade 4)	+/−	+/−	9.76	1.06	0.49	2.16
				−/−	−/−	7.34	0.3	0.7	0.43
3	180	2400		−/−	−/−	8.15	0.7	0.5	1.39
				−/−	−/−	10.09	0.3	0.57	0.52
				−/−	−/−	13.4	0.72	1.79	0.4
				−/−	−/−	21.88	0.79	2.75	0.29
				−/−	−/−	—	—	—	—
				−/−	+/+	10.18	0.71	0.51	1.4

5-FU, 5-fluorouracil; AUC, area under the plasma concentration–time curve.
[a]Grade 4 hematologic and Grades 3–4 non-hematologic toxicities.
[b]This adverse event was dose-limiting toxicity.
[c]Ratio of AUC of SN-38 to AUC of SN-38G.

Phase I Part

The results of the phase I part are shown in Table 2. There was no DLT at dose Level 1. At dose Level 2, one of first three patients had leg edema diagnosed to be caused by deep vein thrombosis (DVT), initially designated as DLT. Three additional patients then received the same dose level. One patient had Grade 3 diarrhea. Although two of the first six patients who received dose Level 2 were initially judged to have DLT, further careful follow-up examinations of the patient considered to have DVT revealed no definite evidence of DVT; this reaction was therefore not considered DLT. We concluded that only one of the six patients given dose Level 2 had DLT and escalated the dose to Level 3. At dose Level 3, no DLT occurred in the first three patients. At that time, we obtained information regarding another ongoing phase I study of FOLFIRI in Japan. In that study, three of six patients who received irinotecan 180 mg/m^2 and infusional 5-FU 3000 mg/m^2 had DLT. This dose level was regarded to be the MTD, and the RD of irinotecan was determined to be the same as our dose Level 3 (14). On the basis of this information, we estimated that the RD was dose Level 3 and decided to confirm this by assigning three additional patients to this dose Level. None of the six patients given dose Level 3 had DLT. We therefore considered dose Level 3 the RD for the next phase II part of this study.

Among the 15 patients participating in the phase I study, the *UGT1A1* genotype was *UGT1A1*1/*1* in 10 patients, *UGT1A1*1/*6* in 2, *UGT1A1*6/*6* in 2 and *UGT1A1*6/*28* in 1. The patient with *UGT1A1*6/*28* genotype had Grade 4 neutropenia after receiving irinotecan at a dose of 150 mg/m^2. The pharmacokinetics of irinotecan, SN-38 and SN-38G were examined in eight patients. The average ratio of the AUC of SN-38 to that of SN-38G (AUC_{SN-38}/AUC_{SN-38G}) in the patients with *UGT1A1*6/*28* and *UGT1A1*6/*6* was 1.78. The average AUC_{SN-38}/AUC_{SN-38G} in the patients with *UGT1A1*1/*1* was 0.65.

Phase II Part

In the phase II part, we evaluated the safety and efficacy of FOLFIRI at the RD in 25 patients: 6 who received the RD in phase I and 19 who were newly enrolled. One patient was homozygous for *UGT1A1*6*, and four were heterozygous for *UGT1A1*6*. No patient harboring the *UGT1A1*28* allele was included.

Twenty-five patients received a median of 4.5 courses (eight sessions) of treatment. Toxic effects occurring during any course of treatment are shown in Table 3. Grade 3 or 4 neutropenia occurred in 11 patients (44%), but only one had febrile neutropenia. Nausea and fatigue were common non-hematologic toxic effects, but most cases were Grade 1 or 2. The dose of irinotecan had to be reduced because of toxicity

Table 3. Toxic effects in the phase II study

	Number of patients				Grade 3 or 4 toxicity (%)
	1	2	3	4	
Hematologic					
Leukopenia	3	11	2	1	12
Neutropenia	1	7	10	1	44
Anemia	4	1	1	0	4
Thrombocytopenia	0	0	0	0	0
Non-hematologic					
Febrile neutropenia	—	—	1	0	4
Nausea	9	9	2	0	8
Vomiting	7	4	1	0	4
Anorexia	7	4	2	0	8
Diarrhea	5	4	1	0	4
Stomatitis	11	1	0	0	0
Fatigue	16	4	1	0	4
Alopecia	10	2	—	—	
Hyperglycemia	0	1	3	0	12

in nine patients (36%); the dose was reduced during the first or second course in eight of these patients. Treatment was delayed during the first two courses in 12 patients (48%). The reasons for treatment delay were neutropenia or leukopenia in 10 patients, anorexia in 2, diarrhea in 2 and infectious colitis in 1. Tumor response is shown in Table 4. Tumor response was assessable in 22 patients. In the other three patients, tumor response could not be assessed because of early discontinuation of treatment. The objective response rate was 24% (95% confidence interval: 9.4–45.1%) with no complete response and six partial responses.

One patient had multiple metastases to the liver, lung and abdominal lymph nodes. The metastases to the lung and abdominal lymph nodes disappeared after eight and a half courses of treatment. In addition, the liver metastasis shrank and could be resected curatively. The major reasons for treatment discontinuation were progressive disease in 18 patients and toxicity or the patient's refusal to continue treatment in 5.

DISCUSSION

This study evaluated the DLT and MTD of the FOLFIRI regimen in Japanese patients with advanced CRC. Observational *UGT1A1* genotyping and pharmacokinetic analysis were also performed in the phase I part, but these lines of information were not reflected for both patient enrollment and dose escalation. We estimated that the RDs of irinotecan and infusional 5-FU for FOLFIRI in Japanese patients were 180 and 2400 mg/m^2, respectively, similar to the RDs in Western countries (1,15).

Table 4. Tumor responses in the phase II study

Response	Number of patients	Percentage
Complete response	0	0
Partial response	6	24
No change	12	48
Progressive disease	4	16
Not evaluated	3	12
Total	25	

The incidence of Grade 3 or 4 neutropenia was higher, but that of diarrhea was lower than the incidences in previous studies conducted in Western countries (Table 3) (1,2). Treatment was frequently delayed because of neutropenia but could be continued after dose reduction. Febrile neutropenia occurred in only one patient in the phase II part. Our results suggest that toxic effects associated with the RD of FOLFIRI as determined in this study were manageable in patients without the *UGT1A1*28* allele.

The doses of irinotecan and infusional 5-FU did not reach the MTD, and only one patient had DLT (Grade 3 diarrhea) at dose Level 2 (irinotecan 150 mg/m^2 and infusional 5-FU 2400 mg/m^2). Therefore, the question remains whether the doses of irinotecan and infusional 5-FU could have been escalated much higher. Previous clinical studies, without *UGT1A1* genotyping, reported that irinotecan could be administered in a dose around 260 mg/m^2. However, these studies did not show a clear advantage of using a higher dose of irinotecan with respect to efficacy and recommended 180–200 mg/m^2 of irinotecan on the basis of toxicity and compliance (15,16).

Although we analyzed *UGT1A1* genotypes, prior stratification had not been applied for dose escalation based on *UGT1A1* genotypes or $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$. *UGT1A1* genotyping and pharmacokinetic data were available for 8 of 12 patients who received dose Levels 2 or 3. The patient with *UGT1A1*6/*28* who had Grade 4 neutropenia during the first course could continue FOLFIRI treatment after reducing the dose of irinotecan to 100 mg/m^2. $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ decreased from 2.16 to 1.56 after dose reduction. The patient had a partial response, with no further severe myelosuppression. One patient with *UGT1A1*1/*1* genotype had DLT. Although there was no clear-cut relation between *UGT1A1* genotype and DLT because of the small number of patients, our results suggest that patients harboring *UGT1A1*6/*28* should be cautiously treated with FOLFIRI and dose reduction might be considered. Previous studies have recommended that caution is exercised when patients with *UGT1A1*6/*28*, **6/*6* or **28/*28* receive FOLFIRI (8–10). However, no confirmatory dose adjustment study in this population exists and optimal dose remains to be explored.

A genotype-driven phase I study of irinotecan included in the FOLFIRI regimen given to Western patients without *UGT1A1*28/*28* demonstrated a higher MTD in those with *UGT1A1*1/*1* or *UGT1A1*1/*28* genotype and a dose-dependent tumor response (17). In Japan and other Asian countries, dose escalation studies of irinotecan in FOLFIRI should be performed taking into account *UGT1A1*28* as well as the **6* genotypes, since the RD may be influenced by the presence of these variant alleles (8–10). The RD of irinotecan for patients without *UGT1A1*28/*28*, **6/*6* or **6/*28* might be higher even among Asians. However, we could not plan the genotype-driven phase I study of irinotecan in the FOLFIRI regimen at that time, because there was limited information regarding the effects of *UGT1A1*6* and **28* on the irinotecan-related toxicities.

In the phase II part of this study, we excluded patients who had at least one *UGT1A1*28* allele, because we had considered at that time that these patients were at a higher risk of irinotecan-induced severe toxicities based on the report by Ando et al. (3). So, we demonstrated the feasibility of FOLFIRI at RDs in a limited population.

The RDs of FOLFIRI in Japanese patients were proved to be consistent with those in Western countries. This finding implies that it may be possible for Japanese patients to participate in global trial(s) to evaluate any investigational new agent combined with FOLFIRI.

In conclusion, this phase I/II study demonstrates that the RDs of irinotecan and infusional 5-FU in FOLFIRI for Japanese patients without the *UGT1A1*28* allele are determined to be 180 and 2400 mg/m^2, respectively. Toxic effects at these doses are manageable based on this protocol setting.

Funding

This study was supported in part by the Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan [21S-8-1], in part by a Grant-in-Aid for Scientific Research (C) from the Ministry of Education [20590546], Culture, Sports, Science and Technology (MEXT) and in part by a Grant-in-Aid for 'Support Project of Strategic Research Center in Private Universities' from the MEXT, awarded by Saitama Medical University Research Center for Genomic Medicine. The study was presented in part at the ASCO GI symposium, San Francisco, CA, January 29–31, 2006.

Conflict of interest statement

None declared.

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0090-9556/11/3902-161–164$20.00
DRUG METABOLISM AND DISPOSITION
Vol. 39, No. 2
35451/3657144
Printed in U.S.A.

DMD 39:161–164, 2011

Short Communication

Delayed Elimination of SN-38 in Cancer Patients with Severe Renal Failure

Received July 13, 2010; accepted October 27, 2010

ABSTRACT:

This prospective study is designed to examine the effects of severe renal failure on the pharmacokinetics of irinotecan. The pharmacokinetics of irinotecan, 7-ethyl-10-hydroxycamptothecin (SN-38), and SN-38 glucuronide (SN-38G) in three cancer patients with severe renal failure [creatinine clearance (Ccr) ≤20 ml/min] who were undergoing dialysis and received 100 mg/m^2 irinotecan as monotherapy were prospectively compared with those in five cancer patients with normal renal function (Ccr ≥60 ml/min). To ensure that the subjects had similar genetic backgrounds of *UDP-glucuronosyltransferase (UGT) 1A1*, patients with *UGT1A1*1/*1*, **1/*6*, or **1/*28* were enrolled. The estimated terminal elimination rate constant of SN-38 in patients undergoing dialysis was approximately one tenth of that in patients with normal renal function (P = 0.025). Approximately 50% of SN-38 was dialyzed with a 2.1-m^2 dialysis membrane, whereas 27% was dialyzed with a 1.5-m^2 membrane. Our results showed that the elimination of SN-38 was significantly delayed in patients with severe renal failure compared with patients with normal renal function. We demonstrated that SN-38 was partly dialyzed.

Introduction

Several lines of evidence have demonstrated that severe renal failure differentially affects drug uptake or efflux transporters and drug-metabolizing enzymes in the liver. Even drugs that are predominantly eliminated by hepatic transport and metabolism can be affected by severe renal failure, leading to unexpected consequences, such as atypical pharmacokinetics and an increased risk of adverse drug reactions. High levels of uremic toxins in such patients are partially implicated in these effects (Nolin et al., 2008).

Irinotecan is extensively metabolized in the liver to an active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), by carboxylesterase, which is then conjugated predominantly by liver UDP-glucuronosyltransferase (UGT) 1A1 to form inactive SN-38 glucuronide (SN-38G) (chemical structures; http://www.pharmgkb.org/search/pathway/irinotecan/metabolites.html). Polymorphisms in *UGT1A1* gene, such as *UGT1A1*28* and **6*, can cause reduced glucuronidation of SN-38, thus resulting in severe irinotecan-induced toxicity. *UGT1A1*6/*6*, **28/*28*, and **6/*28* genotypes have been linked to significantly decreased conversion of SN-38 to SN-38G and severe neutropenia in Asians (Minami et al., 2007).

Transporters expressed in the liver are also implicated in the pharmacokinetics of irinotecan and its metabolites. The uptake of SN-38 from the systemic circulation by hepatocytes is mediated by organic anion transporter peptide 1B1 (OATP1B1) (Nozawa et al., 2005). ATP-binding cassette transporters such as ABCC2, ABCB1, and ABCG2 govern the biliary excretion of irinotecan and its metabolites (http://www.pharmgkb.org/do/serve?objId = PA2001&objCls = Pathway).

Because irinotecan is extensively metabolized and transported in the liver, attention has been focused on the hepatic factors underlying interpatient variability in pharmacokinetics of irinotecan. Studies examining the pharmacokinetics of irinotecan in renally impaired patients are scant. The pharmacokinetics of irinotecan in patients with mild renal impairment who had a creatinine clearance (Ccr) of 35 to 66 ml/min were similar to those in patients with normal renal function (de Jong et al., 2008). Although several case reports have examined the effects of more severe renal dysfunction requiring dialysis on the pharmacokinetics or toxicity of irinotecan (Venat-Bouvet et al., 2007; Czock et al., 2009), no prospective study has been performed; nevertheless, such rare patients are given irinotecan in clinical practice.

Therefore, we prospectively examined the pharmacokinetics of irinotecan, SN-38, and SN-38G in cancer patients with severe renal failure who were undergoing dialysis compared with patients with normal renal function. We enrolled patients with *UGT1A1*1/*1*, **1/*6*, or **1/*28* to ensure that the subjects had similar genetic backgrounds of *UGT1A1*.

Materials and Methods

Materials. Irinotecan, SN-38, and SN-38G were purchased from Toronto Research Chemicals (North York, Canada). All chemicals and solvents were of the highest grade commercially available.

Study Design. Patients who were candidates to receive the 100 mg/m^2 irinotecan monotherapy, satisfying the eligibility criteria listed below, were prospectively enrolled in this study. All patients were divided into two groups:

This work was supported in part by the Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan [Grant 21S-8-1]; and a grant-in-aid for "Support Project of Strategic Research Center in Private Universities" from the Ministry of Education, Culture, Sports, Science and Technology (to Saitama Medical University Research Center for Genomic Medicine).

Article, publication date, and citation information can be found at http://dmd.aspetjournals.org.

doi:10.1124/dmd.110.035451.

ABBREVIATIONS: UGT, UDP-glucuronosyltransferase; SN-38, 7-ethyl-10-hydroxycamptothecin; SN-38G, SN-38 glucuronide; OATP1B1, organic anion transporter peptide 1B1; Ccr, creatinine clearance; CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid; IA, indoleacetic acid; IS, indoxyl sulfate; HA, hippuric acid; λ_z, terminal elimination rate constant.

1) those with a Ccr calculated by the Cockcroft-Gault equation of 60 ml/min or higher and 2) those with a Ccr of 20 ml/min or less who were receiving dialysis. To ensure that the subjects had similar genetic backgrounds of *UGT1A1*, patients with *UGT1A1*1/*1*, **1/*6*, or **1/*28* were enrolled, and patients with *UGT1A1*28/*28*, **6/*6*, or **6/*28* were excluded. The effects of severe renal failure on the pharmacokinetics of irinotecan, SN-38, and SN-38G were studied.

Eligibility. All patients were 20 years or older and had metastatic/recurrent, histologically confirmed solid tumors and an Eastern Cooperative Oncology Group performance status of 0 to 2. No patient had received chemotherapy or radiotherapy within the past 4 weeks. Each patient was confirmed to have adequate bone marrow and liver functions. All patients signed a written informed consent form, granting permission for their peripheral blood samples and medical information to be used for research purposes. The study protocol was approved by the Institutional Review Board of Saitama Medical University.

Treatment. All patients received irinotecan monotherapy as described in its package insert, according to approved usage in Japan. Irinotecan was given at a dose of 100 mg/m^2, either weekly for the first 3 weeks of a 4-week cycle or every 2 weeks. In every 2-week regimen, this lower dose of 100 mg/m^2 was used instead of 150 mg/m^2 at the discretion of the attending physician. Patients with severe renal failure underwent dialysis three times a week and received irinotecan monotherapy on the next day of a dialysis. The interval between the end of the dialysis and the infusion of irinotecan was approximately 17 h.

***UGT1A1* Genotyping.** *UGT1A1*6* and **28* were analyzed using methods as described elsewhere (Araki et al., 2006).

Pharmacokinetic Analysis of Irinotecan and Its Metabolites. Blood samples for pharmacokinetic analysis were obtained at the time of the first dose of irinotecan. The blood samples were taken at the beginning of the irinotecan infusion and 0, 0.25, 0.5, 1, 2, 4, 8, and 24 h after the end of the 1.5-h infusion. Patients with severe renal failure underwent dialysis 1 to 2 h after obtaining the last blood sample. In these patients, blood samples were also taken immediately before starting dialysis, 1 and 2 h after starting dialysis, and immediately after the completion of the dialysis. Total (lactone and carboxylate) plasma concentrations of irinotecan, SN-38, and SN-38G were analyzed by reverse-phase high-performance liquid chromatography (Araki et al., 2006). The plasma concentration-time data of irinotecan and its metabolites were analyzed by a standard noncompartmental method using WinNonlin, version 5.2 software (Pharsight, Mountain View, CA).

Determination of Uremic Toxins. Plasma concentrations of uremic toxins, including 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF), indoxyl sulfate (IS), indoleacetic acid (IA), and hippuric acid (HA), at the beginning of the irinotecan infusion were measured by high-performance liquid chromatography (Nishio et al., 2008).

Statistical Analysis. Pearson's χ^2 test, Fisher's exact test, or the Mann-Whitney *U* test was used to compare patient characteristics between the two groups according to renal function. The Mann-Whitney *U* test was used to analyze differences in pharmacokinetic measurements between the two groups. Differences were considered statistically significant when the two-tailed *P* value was less than 0.05. All analyses were performed with JMP version 7 software (SAS Institute, Inc., Cary, NC).

Results and Discussion

A total of nine Japanese patients with cancer, including three patients with severe renal failure who underwent dialysis, were prospectively enrolled in the present study from May 2005 through April 2010 at Saitama Medical University. During this period, more than 500 patients with cancer received a variety of irinotecan-containing chemotherapy regimens in our university. Among the nine patients enrolled, one patient with normal renal function was excluded because the patient was homozygous for *UGT1A1*6*. The patient characteristics are shown in Table 1.

The elimination of SN-38 was significantly delayed in patients with severe renal failure compared with those without renal failure (Fig. 1A). The terminal elimination rate constant (λ_z) of SN-38 in patients undergoing dialysis [0.00841 ± 0.0037 (mean ± S.D.) 1/h] was approximately one tenth of that in patients with normal renal function (0.0813 ± 0.034 1/h) ($P = 0.025$). No change was observed in the pharmacokinetics of SN-38 in patients who had relatively mild renal failure with a Ccr between 35 and 66 ml/min compared with patients who had normal renal function (de Jong et al., 2008). These results suggest that the severe but mild renal failure causes the alteration of the SN-38 pharmacokinetics. The λ_z estimated for irinotecan and SN-38G were not significantly different between two groups [renal failure versus normal, 0.101 ± 0.0058 versus 0.120 ± 0.045 1/h, $P = 0.070$ (irinotecan); 0.0310 ± 0.014 versus 0.0611 ± 0.034 1/h, $P = 0.18$ (SN-38G)] (Fig. 1, B and C).

TABLE 1

Patient characteristics

Patients	Underwent Dialysis	Normal Renal Function	*P*
Age (year)	67 (56–76)[c]	60 (42–65)	0.24[e]
Sex (male/female)	2/1[d]	3/2	1.0[f]
Performance status (0/1/2)	0/3/0	1/3/1	NA
Tumor type			NA
Ovary	1	2	
Colorectal	1	2	
Gastric	1	0	
Lung	0	1	
Number of prior chemotherapy (1/2/3)	2/0/1	0/5/0	NA
Renal disease			
	Chronic renal failure Diabetic kidney disease Polycystic kidney		
UGT1A1 genotype			
**1/*1*	0	1	NA
**1/*6*	3	2	
**1/*28*	0	2	
Total bilirubin (mg/dl)	0.5 (0.3–0.7)	0.4 (0.3–0.6)	0.44[g]
Serum creatinine (mg/dl)	7.7 (5.3–9.3)	0.68 (0.49–0.99)	0.025
Creatinine clearance (ml/min)[a]	7.09 (6.67–13.3)	82.6 (64.7–124)	0.025
Plasma concentrations of uremic toxins (μM)[b]			0.025
CMPF	81.1 (41.4–90.0)	8.71 (0–20.9)	0.017
Indoxyl sulfate	93.0 (53.3–94.1)	0 (0–12.0)	0.025
Indoleacetic acid	3.07 (2.56–8.00)	1.40 (0–1.53)	0.025
Hippuric acid	80.5 (28.5–144)	4.52 (3.26–6.87)	0.025

NA, not applicable.

[a] Creatinine clearance was calculated with the Cockcroft-Gault equation; [b] measured just before the irinotecan infusion; [c] median (range); [d] number; [e] Pearson χ^2 test. [f] Fisher's exact test; [g] Mann-Whitney *U* test.

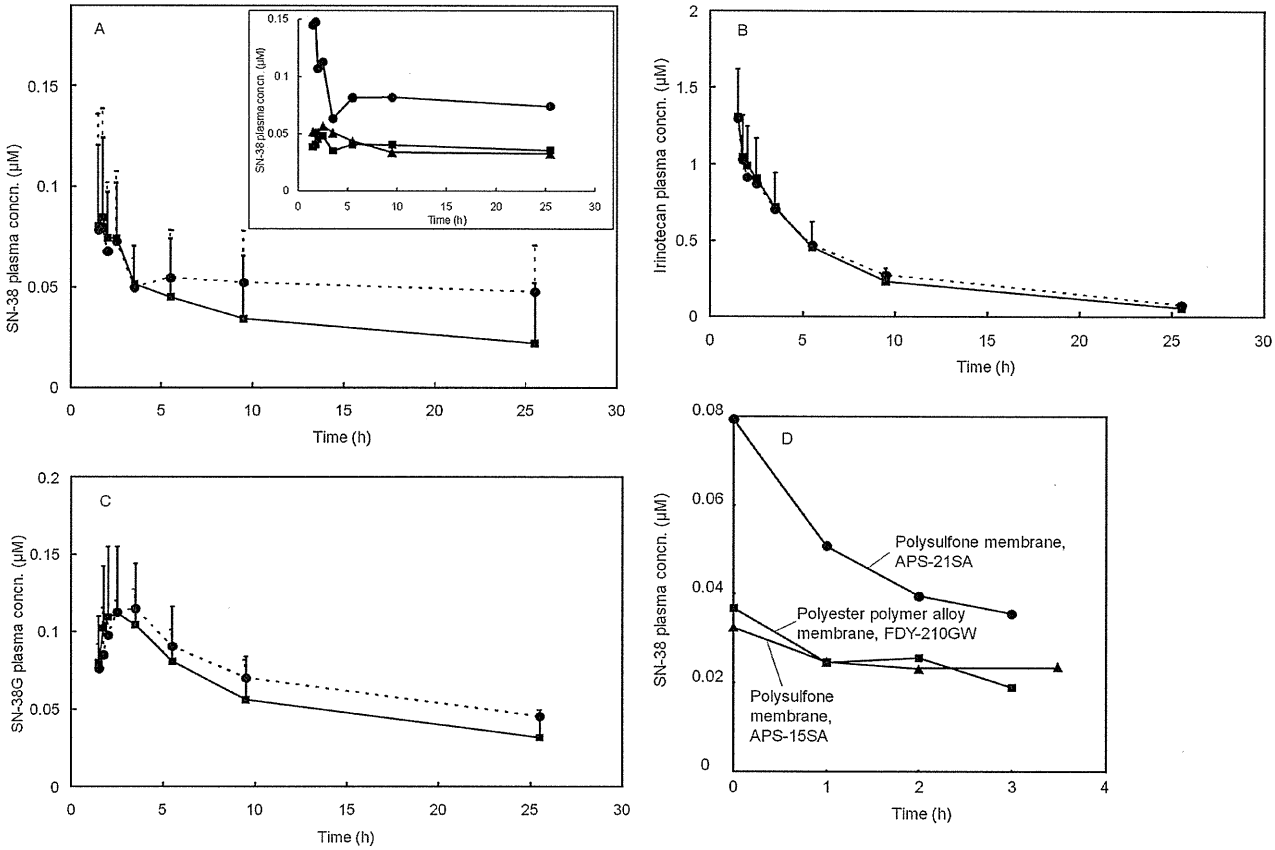

FIG. 1. Pharmacokinetics of SN-38, irinotecan, and SN-38G in patients undergoing dialysis and in those with normal renal function. A, SN-38; B, irinotecan; C, SN-38G; D, pharmacokinetics of SN-38 during dialysis. In A, B, and C, closed circles and dotted lines show the pharmacokinetic data from patients with severe renal failure (n = 3). Closed squares and solid lines indicate the pharmacokinetic data obtained from patients with normal renal function (n = 5). Each data point with error bar represents the mean ± S.D. Time 0 is the start of the irinotecan infusion. Individual pharmacokinetics of SN-38 obtained from three patients with severe renal failure are show in A. Symbols used to represent each of the respective three patients are the same as those used in D. In D, dialysis membranes used for each of the respective three patients are shown.

The mechanism(s) underlying the delayed elimination of SN-38 in patients with insufficient renal function remains speculative. In general, plasma concentrations of uremic toxins increase in parallel to the degree of renal impairment. In our patients, the concentrations of organic anion uremic toxins, such as CMPF, IS, IA, and HA, negatively correlated with Ccr (Table 1). These toxins are substrates of some organic anion transporters. CMPF and IS can directly inhibit OATP1B1 (Sun et al., 2006), which is responsible for the uptake of SN-38 from the systemic circulation by hepatocytes (Nozawa et al., 2005). Therefore, the delayed elimination of SN-38 in patients with severe renal failure might be attributed to the inhibition of OATP1B1 by these uremic toxins. Because ATP-binding cassette transporters involved in the efflux of SN-38 can transport organic anions, CMPF, IS, IA, and HA might serve as substrates of them, thereby inhibiting the efflux of SN-38, thus leading to the delayed elimination of SN-38. The elimination half-life of cerivastatin, a substrate of the nonrenal ABCB1, OATP, ABCC, and ABCG2, is approximately 1.5 times prolonged in patients with kidney disease (Nolin et al., 2008), indirectly supporting our hypothesis.

The significantly delayed elimination was observed only for SN-38, but not for SN-38G. All patients tested were likely to have similar glucuronidation capacity for SN-38, because they possessed *UGT1A1*1/*1*, **1/*6*, or **1/*28*. Uremic toxins measured in the present study only slightly inhibited the activity of UGT1A1-mediated SN-38 glucuronidation in vitro (data not shown). Given that, SN-38 glucuronidation may be similar between patients with and without severe renal failure. Therefore, the modification of transporter(s) responsible for SN-38 or SN-38G by a high concentration of uremic toxins in patients with severe renal dysfunction may cause the pharmacokinetic profiles of SN-38 and SN-38G. However, further studies are needed to clarify the mechanism.

Patients with severe renal failure underwent dialysis 1 to 2 h after the last blood sampling. Plasma concentration of SN-38 determined at 24 h after the end of irinotecan infusion and that measured immediately before the start of dialysis (1–2 h after the 24-h blood sampling) for each patient was almost equal, indicating that the λz of SN-38 seen at this period was nearly equal to zero. Assuming that the λz of SN-38 during the dialysis was nearly zero, approximately 50% of SN-38 was dialyzed in patients who received dialysis with a 2.1-m^2 polysulfone membrane APS-21SA (Asahi Kasei Kuraray Medical, Tokyo, Japan) or a polyester polymer alloy membrane FDY-210GW (Nikkiso, Tokyo, Japan) (Fig. 1D). SN-38 was dialyzed by 27% in a patient who underwent dialysis with a 1.5-m^2 polysulfone membrane APS-15SA (Asahi Kasei Kuraray Medical) (Fig. 1D). In contrast, SN-38 was not dialyzable in previous studies (Venat-Bouvet et al., 2007; Czock et al., 2009), but they did not mention the specifications of the dialyzer used. There may be differences between the specifications of dialyzers used in this study and previous studies.

All patients with severe renal failure suffered from grade 2, 3, or 4 neutropenia (National Cancer Institute Common Toxicity Criteria for Adverse Events, Version 3.0), even though dialyzes were performed. Grade 2 or 3 neutropenia was prolonged in two of these patients. The prolonged neutropenia resulted in the delay of the second irinotecan treatment until 24 or 34 days after the initial infusion. In contrast, no delay of the second irinotecan treatment caused by neutropenia was observed in patients with normal renal function. The delayed elimination of SN-38 may be one of the causes of prolonged neutropenia. If so, dialysis can be started earlier than 24 h after irinotecan infusion to lower the plasma SN-38 concentration. Alternatively, irinotecan infusion should be performed just after finishing the dialysis to minimize the effects of uremic toxins, if the delayed elimination of SN-38 is truly caused by uremic toxins. However, it should be necessary to further optimize the dialysis conditions, including the specification of the dialyzer, and the timing and duration of the dialysis for the better management of neutropenia in patients with severe renal failure.

In conclusion, the elimination of SN-38 in patients with severe renal failure was significantly delayed compared with that in patients with normal renal function. The SN-38 was in part dialyzed.

Authorship Contributions

Participated in research design: Fujita and Sasaki.

Conducted experiments: Akiyama and Sugiyama.

Contributed new reagents or analytic tools: Fujita.

Performed data analysis: Fujita, Kawara, Saji, Narabayashi, Ando, and Hirose.

Wrote or contributed to the writing of the manuscript: Fujita and Sasaki.

Other: Sunakawa, Miwa, Ishida, Yamashita, Mizuno, Ichikawa, Yamamoto, Nagashima, and Miya enrolled and followed patients, and Sasaki acquired funding for the research.

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JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2011;41(5)617–623
doi:10.1093/jjco/hyr010
Advance Access Publication 9 February 2011

Regimen Selection for First-line FOLFIRI and FOLFOX Based on *UGT1A1* Genotype and Physical Background is Feasible in Japanese Patients with Advanced Colorectal Cancer

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Received September 15, 2010; accepted January 14, 2011

Objective: We examined the feasibility of regimen selection for first-line irinotecan, 5-fluorouracil and leucovorin or oxaliplatin, 5-fluorouracil and leucovorin in Japanese patients with advanced colorectal cancer based on *UDP-glucuronosyltransferase 1A1* genotype as well as physical status of patients related to diarrhea.

Methods: As first-line irinotecan, 5-fluorouracil and leucovorin is a little bit superior to oxaliplatin, 5-fluorouracil and leucovorin with respect to efficacy and toxicity, patients without risk factors of irinotecan-induced toxicity were first assigned to irinotecan, 5-fluorouracil and leucovorin. Patients with *UDP-glucuronosyltransferase 1A1*28/*28*, **6/*6*, **28/*6* or **28/*27* and those with ascites, peritoneal dissemination or diarrhea first received oxaliplatin, 5-fluorouracil and leucovorin to avoid the irinotecan-induced neutropenia and diarrhea, respectively. We retrospectively evaluated the feasibility of this strategy by assessing toxicity and total progression-free survival in first- and subsequent second-line therapies in all patients studied.

Results: In the first-line irinotecan, 5-fluorouracil and leucovorin ($n = 61$), Grade 4 neutropenia, febrile neutropenia and Grade 3 diarrhea occurred in 8.2, 3.3 and 3.3% of patients, respectively. In the first-line oxaliplatin, 5-fluorouracil and leucovorin ($n = 26$), Grade 4 neutropenia, febrile neutropenia, Grade 3 thrombocytopenia and Grade 3 neuropathy were observed in 11.5, 3.8, 3.8 and 7.7% of patients, respectively. In the second-line oxaliplatin, 5-fluorouracil and leucovorin ($n = 38$), Grade 3 diarrhea occurred in 2.6% of patients. In the second-line irinotecan monotherapy ($n = 11$), Grade 4 or febrile neutropenia occurred in 18% of patients and Grade 3 diarrhea in 9.1% of patients. In second-line S-1 ($n = 9$), Grade 3 anemia occurred in 2 patients. Median total progression-free survival in all 87 patients was 11.5 months.

Conclusions: Present regimen selection strategy would be feasible, since it causes less toxicity and similar efficacy comparing to previous studies. Determination of appropriate reduced dose in the second-line irinotecan monotherapy or other standard second-line therapy for patients with high-risk to irinotecan-induced toxicity might make this strategy more effective.

Key words: FOLFIRI – FOLFOX – physical condition – regimen selection – UGT1A1 genotyping

INTRODUCTION

Irinotecan is a camptothecin derivative that exerts cytotoxic effects by inhibiting topoisomerase I. This drug has been approved for the treatment of a wide variety of solid tumors, including colorectal cancer. However, patients and oncologists are deeply concerned about the dose-limiting toxic effects of irinotecan, such as myelosuppression and delayed-type diarrhea (1–3). Combined therapy with irinotecan, 5-fluorouracil (5-FU) and leucovorin (LV) (FOLFIRI) has been proven to be highly effective for the first-line treatment of patients with advanced colorectal cancer (4). The combination of oxaliplatin, 5-FU and LV (FOLFOX) is also a standard first-line regimen for advanced colorectal cancer (5). These regimens provide similar survival benefits, but have different toxicological profiles, depending mainly on the use of irinotecan or oxaliplatin. Furthermore, FOLFIRI followed by FOLFOX is associated with slightly, but not significantly longer survival than FOLFOX followed by FOLFIRI. In addition, the response rate of FOLFOX is superior to that of FOLFIRI when these regimens are used as the second-line therapy (5). Taking these lines of evidence into consideration, FOLFIRI is superior to FOLFOX as first-line treatment for patients with advanced colorectal cancer, if the patients do not have backgrounds, which are related to irinotecan-induced severe neutropenia or diarrhea.

Previously, physicians predicted the irinotecan-induced adverse events in FOLFIRI according to only physical conditions of patients with advanced colorectal cancer. Physicians tended not to use FOLFIRI as the first-line therapy for patients with ascites, peritoneal dissemination or diarrhea to avoid severe diarrhea induced by irinotecan. On the other hand, there have been no predictive markers of irinotecan-related severe neutropenia.

Several studies have linked *UDP-glucuronosyltransferase* (*UGT*) *1A1*28* genotype to irinotecan-related neutropenia. Patients homozygous for *UGT1A1*28* have a significantly higher risk of severe neutropenia due to irinotecan than those who do not possess this genotype (6, 7), because *UGT1A1*28* decreases UGT1A1 protein expression and reduces glucuronidation capacity for SN-38. In Asians, a specific mutation, *UGT1A1*6* (8), has been proven to reduce the catalytic activity of UGT1A1 (9, 10). The *UGT1A1*28/*28*, **6/*6* and **6/*28* genotypes have been shown to be related not only to a lower ratio of the area under the plasma concentration–time curve of SN-38G to that of SN-38, but also to severe neutropenia in Asian populations (11–13). Compound heterozygotes of *UGT1A1*28* and *UGT1A1*27* seen in Japanese were also suggested to be related to severe neutropenia of irinotecan (6). Thus, the *UGT1A1* genotyping was established as predictive marker for irinotecan-induced severe neutropenia and was approved not only by the Food and Drug Administration in the USA but also by the Ministry of Health, Labour and Welfare of Japan.

Given that, we established a strategy for the regimen selection of FOLFIRI as the first-line therapy for patients with advanced colorectal cancer, aiming to avoid the irinotecan-induced severe toxicities that are related to the reduced dose intensity of irinotecan (Fig. 1). We considered the *UGT1A1* genetic testing in addition to the clinical physical status of patients to select FOLFIRI or FOLFOX regimen. Patients with *UGT1A1*28/*28*, **6/*6*, **28/*6* or **28/*27* first received FOLFOX to avoid the irinotecan-induced severe neutropenia. Patients who had the risk factor of irinotecan-induced severe diarrhea including ascites, peritoneal dissemination and diarrhea also first received FOLFOX, even though they possessed *UGT1A1*1/*1*, **1/*6* or **1/*28* genotypes. Patients with *UGT1A1*1/*1*, **1/*28* or **1/*6* and without the risk factor of irinotecan-induced severe diarrhea received first-line FOLFIRI.

To evaluate the feasibility of this regimen selection strategy for first-line FOLFIRI and FOLFOX, we retrospectively assessed toxicity and efficacy in first-line and subsequent second-line chemotherapies in all patients studied.

PATIENTS AND METHODS

Patients

All patients with a histologically confirmed diagnosis of advanced colorectal cancer who had an Eastern Cooperative Oncology Group performance status of 0–2, adequate bone marrow, liver and renal functions and no history of chemotherapy for advanced disease were eligible. Patients with diarrhea of four times a day or more were excluded. Any previous adjuvant chemotherapy must have been completed at least 6 months before treatment. All patients signed

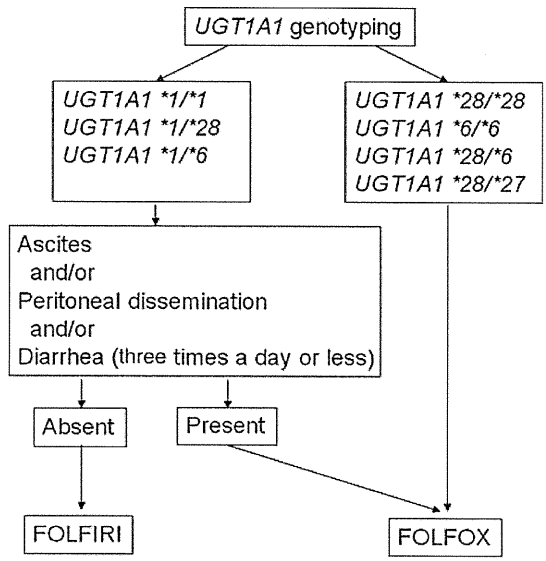

Figure 1. *UGT1A1* genotype-based strategy for the selection of FOLFIRI or FOLFOX as the first-line therapy in patients with advanced colorectal cancer.

written informed consent for their peripheral blood samples to be used for *UGT1A1* genotyping. The protocols of this retrospective study and *UGT1A1* genotyping were separately approved by the Institutional Review Board of Saitama Medical University.

Study Design

Patients with *UGT1A1*28/*28*, **6/*6*, **28/*6* or **28/*27* were considered high-risk group of irinotecan-induced severe neutropenia. Patients with ascites as judged by computed tomography or ultrasonography, hisotologically or cytologically confirmed peritoneal dissemination or diarrhea of three times a day or less were considered as high-risk group of irinotecan-induced severe diarrhea. These patients received FOLFOX as the first-line therapy. Other patients with *UGT1A1*1/*1*, **1/*28* or **1/*6* and without the risk factors for irinotecan-related diarrhea received FOLFIRI as the first-line therapy.

First-Line FOLFIRI and FOLFOX Treatments

The FOLFIRI regimen comprised a 2-h intravenous infusion of irinotecan (150 or 180 mg/m^2) and LV (200 mg/m^2) on Day 1, followed by an intravenous bolus injection of 5-FU (400 mg/m^2) and a 46-h intravenous infusion of 5-FU (2400 mg/m^2), repeated every 2 weeks. The FOLFOX regimen comprised a 2-h intravenous infusion of oxaliplatin (85 mg/m^2) and LV (200 mg/m^2), followed by an intravenous bolus injection of 5-FU (400 mg/m^2) and a 46-h intravenous infusion of 5-FU (2400 mg/m^2), repeated every 2 weeks.

Second-Line Treatments

FOLFOX was given by the same method as the first-line treatment. Irinotecan monotherapy regimen comprised a 1.5-h intravenous infusion of irinotecan (150 mg/m^2), repeated every 2 weeks. S-1 was given per oral twice daily for 28 consecutive days, followed by 2 weeks of rest. The dose of S-1 was fixed based on the patients' body surface area (BSA) according to the manufacturer's package insert as distributed in Japan. The dose was 80 mg/day for patients with a BSA of <1.25 m^2, 100 mg/day for those with a BSA of 1.25–1.5 m^2 and 120 mg/day for those with a BSA of >1.5 m^2.

Evaluation of Efficacy and Toxicity

Toxicity was assessed according to the National Cancer Institute common terminology criteria for adverse events, version 3.0 (http://ctep.cancer.gov/reporting/ctc_v30.html). Tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors (http://www.recist.com/index.html) for at least 2 months by computed tomography imaging or ultrasonography. Efficacy was evaluated on the basis of the overall response rate and progression-free survival (PFS). PFS was defined as the date of starting treatment with FOLFIRI, FOLFOX or second-line chemotherapies to the date of disease progression as defined by the RECIST criteria or the date of death from any cause. The same imaging method was used for baseline tumor measurements and tumor reassessments. Total PFS was defined as the summation of PFSs in first- and second-line chemotherapies observed in respective patients. When patients did not receive second-line chemotherapy, the total PFS was equal to the PFS in first-line chemotherapy.

UGT1A1 Genotyping

Genomic DNA was extracted from 200 μl of peripheral blood, which had been stored at −80°C until analysis, with the use of a QIAamp Blood Kit (QIAGEN GmbH, Hilden, Germany). Two polymorphisms [G71R (**6*) and P229Q (**27*)] were analyzed by the polymerase chain reaction-restriction fragment length polymorphism method, as described elsewhere (14). The TATA box polymorphism (**28*) was determined by the direct sequencing method, as described by Fujita *et al.* (14).

Statistical Analysis

Total PFS was calculated by the Kaplan–Meier method. The analysis was conducted using JMP version 6 software (SAS Institute, Inc., Cary, NC).

Assessment of Feasibility of the Present Regimen Selection

The feasibility of the selection strategy for first-line FOLFIRI or FOLFOX was assessed by toxicity and efficacy in all patients: (1) Frequencies of typical toxicity(ies) for first-line FOLFIRI and FOLFOX, and the second-line chemotherapies were equal to or less than those observed in representative previous studies; (2) Total PFS in first- and subsequent second-line chemotherapies observed in all patients studied was almost equal to that in representative previous studies.

RESULTS

Patient Characteristics

A total of 112 patients with advanced colorectal cancer received first-line chemotherapy from June 2003 through April 2008. Chemotherapeutic regimens given to all of the patients are shown in Table 1. First-line FOLFIRI was given to 61 patients and FOLFOX to 26. These 87 patients were studied. Patient characteristics are shown in Table 2. Six patients received first-line FOLFOX based on *UGT1A1* genotypes and 20 patients received FOLFOX according to their physical conditions to avoid irinotecan-induced toxicity.

Table 1. First-line chemotherapy performed for advanced colorectal cancer patients in our institute from June 2003 to April 2008

Regimen	Number of patients ($n = 112$)	%
FOLFIRI	61	54
FOLFOX	26	23
5-FU/LV	8	7
Irinotecan monotherapy	5	5
FOLFOXIRI	4	4
IFL	4	4
S-1	4	4

FOLFIRI, irinotecan plus 5-fluorouracil and leucovorin; FOLFOX, oxaliplatin plus 5-fluorouracil and leucovorin; 5-FU/LV, 5-fluorouracil plus leucovorin; FOLFOXIRI, oxaliplatin plus iriontecan plus 5-fluorouracil and leucovorin; IFL, irinotecan plus 5-fluorouracil and leucovorin.

Table 2. Patient characteristics

	FOLFIRI ($n = 61$)	FOLFOX ($n = 26$)
Gender, *n* (%)		
Male	38 (62)	12 (46)
Female	23 (38)	14 (54)
Age (years)		
Median (range)	59 (39–74)	62 (38–79)
ECOG PS, *n* (%)		
0	38 (62)	15 (58)
1	23 (38)	11 (42)
Total bilirubin level (mg/dl)		
Median (range)	0.5 (0.2–1.1)	0.6 (0.3–1.4)
Serum creatinine level (mg/dl)		
Median (range)	0.64 (0.39–1.27)	0.77 (0.41–1.54)
Primary tumor site, *n* (%)		
Colon	51 (84)	18 (69)
Rectum	10 (16)	8 (31)
UGT1A1 genotype, *n* (%)		
**1/*1*	43 (70)	9 (34)
**1/*6*	16 (26)	8 (30)
**1/*28*	2 (4)	3 (12)
**6/*6*	0 (0)	3 (12)
**28/*6*	0 (0)	1 (4)
**28/*27*	0 (0)	2 (8)
Patients assigned to FOLFOX		
UGT1A1 genotype		6 (23)
Peritoneal dissemination		15 (58)
Diarrhea		5 (19)

ECOG, Eastern Cooperative Oncology Group; PS, performance status. *UGT1A1, UDP-glucuronosyltransferase 1A1.*

Toxicity in First-line Treatments

The main adverse events associated with first-line FOLFIRI or FOLFOX are presented in Table 3. In FOLFIRI, Grade 4 neutropenia occurred in 5 (8.2%) patients. Febrile neutropenia and Grade 3 diarrhea were seen in 2 (3.3%) patients. In FOLFOX, Grade 4 neutropenia occurred in 3 (11.5%) patients. Febrile neutropenia and Grade 3 thrombocytopenia were observed in one patient (3.8%). Grade 3 neuropathy occurred in 2 (7.7%) patients. However, no other Grade 3 or 4 non-hematological adverse events occurred in FOLFOX. No patient who harbored *UGT1A1*6/*6*, **28/*6* or **28/*27* receiving FOLFOX had Grade 4 neutropenia or other toxic effects of Grade 3 or higher. The discontinuation of FOLFIRI or FOLFOX due to toxicity were 3 (4.9%) and 5 (19%) patients, respectively. There were no treatment-related deaths in both groups.

Efficacy in First-line Chemotherapies

The efficacy of first-line FOLFIRI or FOLFOX was evaluated on the basis of the overall response rate and PFS (Table 4). The overall response rates were 43% in FOLFIRI and 46% in FOLFOX (Table 3). Median PFS was 7.5 months in FOLFIRI and was 8.7 months in FOLFOX. The median number of FOLFIRI and FOLFOX treatments were 7.0 (range of 1–38) and 6.5 (range of 1–18), respectively.

Second-line Chemotherapies

Among the patients who received first-line FOLFIRI, 38 patients (62%) received second-line FOLFOX and 4 (7%) S-1. The remaining 19 (31%) did not receive any second-line chemotherapies (10 others including surgery or radiotherapy and 9 best supportive care). In second-line FOLFOX, no Grade 4 or febrile neutropenia was observed. Grade 3

Table 3. Toxicity in patients treated with first-line FOLFIRI or FOLFOX

		FOLFIRI ($n = 61$)				FOLFOX ($n = 26$)			
Toxicity	Grade	1	2	3	4	1	2	3	4
		n	*n*	*n*	*n*	*n*	*n*	*n*	*n*
Neutropenia		4	14	12	5	0	5	9	3
Febrileneutropenia		0	0	2	0	0	0	1	0
Anemia		37	7	2	0	4	10	0	0
Thrombocytopenia		6	0	0	0	12	1	1	0
Nausea		31	14	3	0	15	1	0	0
Vomiting		19	6	3	0	6	1	0	0
Diarrhea		8	6	2	0	4	1	0	0
Neuropathy		3	0	0	0	19	3	2	0
Hypersensitivity		0	0	0	0	2	1	0	0

Table 4. Response rate and progression-free survival in patients treated with first-line FOLFIRI or FOLFOX

	FOLFIRI ($n = 61$)	FOLFOX ($n = 26$)
	n (%)	*n* (%)
Response		
CR	0 (0)	0 (0)
PR	26 (43)	12 (46)
SD	20 (33)	12 (46)
PD	10 (16)	1 (4)
NE	5 (8)	1 (4)
Overall response rate		
% of patients	43	46
Progression-free survival		
Median (months)	7.5	8.7
Range	0.9–20.0	1.5–28.3

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable.

anemia and diarrhea occurred in respective one patient (2.6%). However, no other Grade 3 or 4 adverse events occurred. The overall response rate and median PFS in the second-line FOLFOX were 13% and 5.4 months, respectively. In second-line S-1, Grade 3 anemia occurred in one patient. No other Grade 3 or 4 adverse events occurred. The overall response rate and median PFS in second-line S-1 were 0% and 0.8 months, respectively.

In patients treated with first-line FOLFOX, 11 patients (42%) received second-line irinotecan monotherapy, 5 (19%) S-1 and 10 (38%) best supportive care. In second-line irinotecan monotherapy group, Grade 4 or febrile neutropenia was observed in respective 2 patients (18%). Grade 3 anemia and Grade 3 thrombocytopenia occurred in 2 (18%) and 1 patient (9.1%), respectively. Grade 3 diarrhea occurred in 1 patient (9.1%). The overall response rate and median PFS in the second-line irinotecan monotherapy were 0% and 2.0 months, respectively. In the second-line S-1, Grade 3 anemia occurred in one patient. No other Grade 3 or 4 adverse events were observed. The overall response rate and median PFS in second-line S-1 were 0% and 1.5 months, respectively.

Among six patients with *UGT1A1*6/*6*, **28/*6* or **28/*27* who received first-line FOLFOX, three received second-line irinotecan monotherapy, one was given S-1 and others received best supportive care. Irinotecan therapy was started with the standard dose of 150 mg/m^2 in Japan, because there has been no information regarding the optimal reduced dose of irinotecan for patients possessing these *UGT1A1* genotypes. Among three patients given second-line irinotecan monotherapy, two patients experienced respective Grade 3 or 4 neutropenia and one patient Grade 3 diarrhea. The irinotecan doses in these patients for the next courses were reduced by the physicians in charge.

Total PFS in First- and Second-line Therapies in All Patients Examined

The median total PFS in all 87 patients studied was 11.5 months (Fig. 2).

DISCUSSION

This is the first study to select the first-line FOLFIRI or FOLFOX regimen by considering *UGT1A1* genetic testing in addition to physical conditions in patients with advanced colorectal cancer. The feasibility of this strategy was evaluated as follows:

1. The toxicities observed during the all first- and second-line chemotherapies were compared with those observed in representative studies.

In patients treated with first-line FOLFIRI, the frequency of Grade 4 neutropenia was slightly lower than that previously reported (9%) (4, 5, 15). The frequencies of febrile neutropenia and Grade 3 diarrhea were lower than those reported previously (febrile neutropenia, 7% and Grade 3–4 diarrhea, 14%) (4, 5, 15). The patient selection for FOLFIRI adopted in the present strategy appears to be effective to reduce the irinotecan-induced toxicities. In the first-line FOLFOX, the frequencies of Grade 4 neutropenia, Grade 3 thrombocytopenia and Grade 3 neuropathy were lower than those reported previously (Grade 4 neutropenia, 13%; Grade 3 thrombocytopenia, 5% and Grade 3 neuropathy, 34%) (4, 5, 15). Patients who were assigned to FOLFOX because of ascites, peritoneal dissemination and diarrhea did not suffer from Grade 3 or higher gastrointestinal adverse events such as nausea, vomiting and diarrhea, which were relatively often observed in FOLFIRI. Furthermore, no patient who harbored *UGT1A1*6/*6*, **28/*6* or **28/*27* receiving

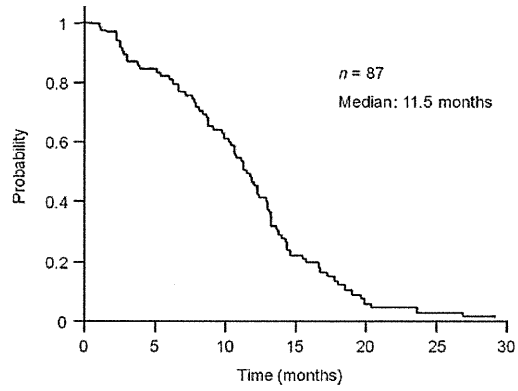

Figure 2. Kaplan–Meier analysis of total progression-free survival in all 87 patients.

FOLFOX had Grade 4 neutropenia or other toxic effects of Grade 3 or higher.

In second-line FOLFOX, the frequencies of Grade 3 or 4 neutropenia, Grade 3 thrombocytopenia and Grade 3 neuropathy were lower than those reported previously (Grade 4 neutropenia, 17%; Grade 3 thrombocytopenia, 1% and Grade 3 neuropathy, 20%) (5). The frequency of toxicities seen in the second-line irinotecan monotherapy was compared with that in the second-line FOLFIRI (5), since (i) there have been few studies of second-line irinotecan monotherapy with large number of patients and (ii) we can evaluate the toxicity more severely in second-line irinotecan monotherapy, because FOLFIRI is stronger than irinotecan monotherapy in terms of toxicity. The frequencies of Grade 3 or 4 neutropenia and febrile neutropenia seen in second-line irinotecan monotherapy were higher than those previously reported (Grade 3 or 4 neutropenia, 21% and febrile neutropenia, 1%) (5), while the frequency of Grade 3 diarrhea was similar to that reported previously (8%) (5). Because patients who had the risk factor for irinotecan-induced severe toxicity received second-line irinotecan monotherapy, frequencies of severe neutropenia and febrile neutropenia were higher than that previously reported. In second-line S-1, the frequency of Grade 3 anemia was similar to that reported in previous study (16). The frequencies of non-hematological toxicities such as nausea, diarrhea and mucositis were lower than those reported previously (16).

Collectively, the present regimen selection strategy appears to be feasible in terms of toxicities, except for the patients with risk for irinotecan-induced toxicity who received the second-line irinotecan monotherapy. Appropriate reduced dose should be determined and other chemotherapies without irinotecan should be developed for these patients.

2. The median total PFS in all 87 patients evaluated was 11.5 months (Fig. 2). We compared this clinical outcome during first- and second-line treatments with duration of disease control (DDC) used in OPTIMOX studies, which collected the data until second-line therapy (17, 18), since the definition of DDC is almost equal to that of our total PFS. In OPTIMOX studies, DDC was defined as PFS in first-line FOLFOX and maintenance with simplified 5-FU and LV regimen plus PFS of FOLFOX reintroduction (17, 18). The median total PFS of 11.5 months in our study was almost similar to that reported in OPTIMOX studies (10.6–13.1 months) (17, 18).

Taking these considerations into account, the regimen selection of the first-line FOLFIRI or FOLFOX therapy based on the *UGT1A1* genotyping in addition to patient physical conditions that are related to irinotecan-induced toxicity might be feasible, since it causes less toxicity and similar efficacy comparing to previous studies. Determination of appropriate reduced dose in second-line irinotecan monotherapy or other standard second-line therapy for patients with high risk to irinotecan-induced toxicity might make this strategy more effective.

Previous studies of first-line FOLFIRI therapy in patients with advanced colorectal cancer have reported the response rate of 31–56% and PFS of 8.5 months (4, 5, 15). First-line FOLFOX therapy showed the similar efficacy as FOLFIRI (response rate, 34–54% and PFS, 8.0 months) (5, 15, 19). In our study, the response rate and PFS in patients assigned to FOLFIRI or FOLFOX were comparable to those reported previously. It should be noted that the response rate and PFS [50% and 8.6 months (range of 2.5–15.2)] seen in patients who received FOLFOX because of harboring *UGT1A1*6/*6*, **28/*6* or **28/*27* genotype were not statistically significantly different from those observed in patients assigned to FOLFIRI.

To further confirm the present results, the prospective study involving larger numbers of patients should be planned to confirm our data, even though many patients are now treated with FOLFIRI or FOLFOX combined with monoclonal antibodies such as bevacizumab or cetuximab as the first-line therapy for advanced colorectal cancer in Japan (20–23).

At present, there has been no evidence whether or not the present strategy is applicable when FOLFIRI or FOLFOX are used in combination with bevacizumab or cetuximab. Further studies are necessary to confirm this point.

If the optimal reduced dose(s) of irinotecan can be determined for patients who have a high risk of irinotecan-induced neutropenia because of *UGT1A1*28/*28*, **6/*6*, **28/*6* or **28/*27* genotype, *UGT1A1* genotyping should become essential not only for regimen selection but also for dose decision-making.

In summary, our results demonstrate that the selection of first-line FOLFIRI or FOLFOX in patients with advanced colorectal cancer based on *UGT1A1* genotyping in addition to patient physical condition would be feasible, since it causes less toxicity and similar efficacy comparing to previous studies. Determination of appropriate reduced dose in second-line irinotecan monotherapy or other standard second-line therapy for patients with high risk to irinotecan-induced toxicity might make this strategy more effective. Severe irinotecan-induced neutropenia in first-line FOLFIRI was avoided in patients with *UGT1A1*28/*28*, **6/*6*, **28/*6* or **28/*27* by assigning these patients to first-line FOLFOX. This strategy of regimen selection for first-line FOLFIRI and FOLFOX might be feasible.

Funding

This study was supported in part by the Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan (21S-8-1), and in part by a grant-in-aid for 'Support Project of Strategic Research Center in Private Universities' from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) to Saitama Medical University Research Center for Genomic Medicine.

Conflict of interest statement

None declared.

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