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.

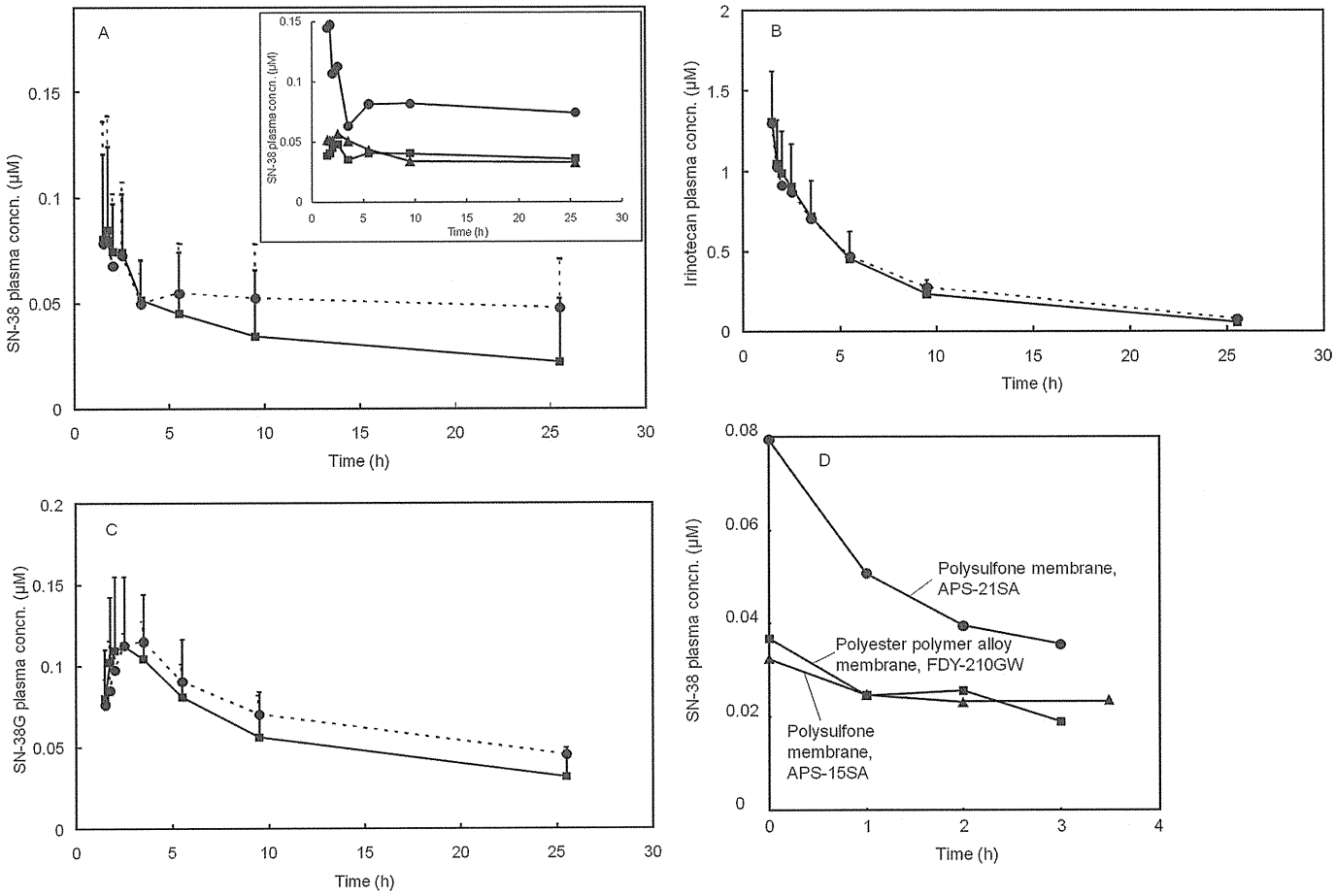

FIG. 1. Pharmacokinetics of SN-38, irinotecan, and SN-38G in patients undergoing dialysis and in those with normal renal function. A, SN-38; B, irinotecan; C, SN-38G; D, pharmacokinetics of SN-38 during dialysis. In A, B, and C, closed circles and dotted lines show the pharmacokinetic data from patients 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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References

Araki K, Fujita K, Ando Y, Nagashima F, Yamamoto W, Endo H, Miya T, Kodama K, Narabayashi M, and Sasaki Y (2006) Pharmacogenetic impact of polymorphisms in the coding region of the UGT1A1 gene on SN-38 glucuronidation in Japanese patients with cancer. *Cancer Sci* **97:**1255–1259.

Czock D, Rasche FM, Boesler B, Shipkova M, and Keller F (2009) Irinotecan in cancer patients with end-stage renal failure. *Ann Pharmacother* **43:**363–369.

de Jong FA, van der Bol JM, Mathijssen RH, van Gelder T, Wiemer EA, Sparreboom A, and Verweij J (2008) Renal function as a predictor of irinotecan-induced neutropenia. *Clin Pharmacol Ther* **84:**254–262.

Minami H, Sai K, Saeki M, Saito Y, Ozawa S, Suzuki K, Kaniwa N, Sawada J, Hamaguchi T, Yamamoto N, et al. (2007) Irinotecan pharmacokinetics/pharmacodynamics and UGT1A genetic polymorphisms in Japanese: roles of UGT1A1*6 and *28. *Pharmacogenet Genomics* **17:**497–504.

Nishio T, Takamura N, Nishii R, Tokunaga J, Yoshimoto M, and Kawai K (2008) Influences of haemodialysis on the binding sites of human serum albumin: possibility of an efficacious administration plan using binding inhibition. *Nephrol Dial Transplant* **23:**2304–2310.

Nolin TD, Naud J, Leblond FA, and Pichette V (2008) Emerging evidence of the impact of kidney disease on drug metabolism and transport. *Clin Pharmacol Ther* **83:**898–903.

Nozawa T, Minami H, Sugiura S, Tsuji A, and Tamai I (2005) Role of organic anion transporter OATP1B1 (OATP-C) in hepatic uptake of irinotecan and its active metabolite, 7-ethyl-10-hydroxycamptothecin: in vitro evidence and effect of single nucleotide polymorphisms. *Drug Metab Dispos* **33:**434–439.

Sun H, Frassetto L, and Benet LZ (2006) Effects of renal failure on drug transport and metabolism. *Pharmacol Ther* **109:**1–11.

Vénat-Bouvet L, Saint-Marcoux F, Lagarde C, Peyronnet P, Lebrun-Ly V, and Tubiana-Mathieu N (2007) Irinotecan-based chemotherapy in a metastatic colorectal cancer patient under haemodialysis for chronic renal dysfunction: two cases considered. *Anticancer Drugs* **18:**977–980.

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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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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

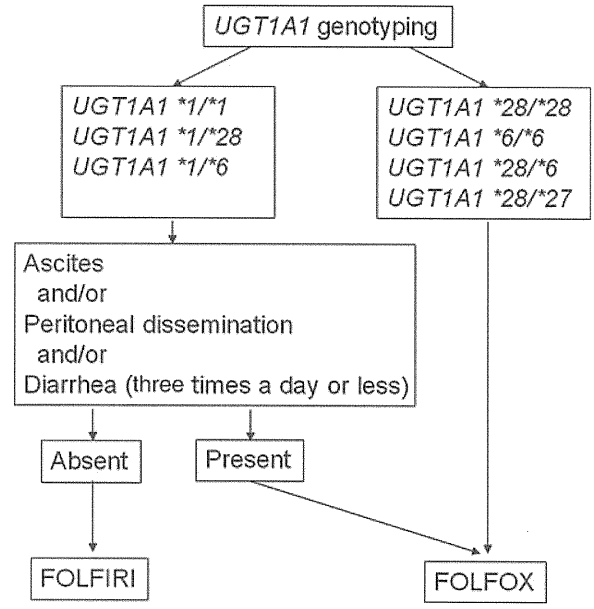

Figure 1. *UGT1A1* genotype-based strategy for the selection of FOLFIRI or FOLFOX as the first-line therapy in patients with advanced colorectal cancer.

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

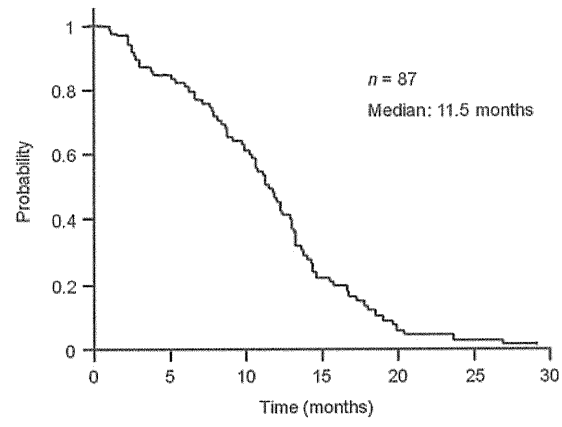

Figure 2. Kaplan–Meier analysis of total progression-free survival in all 87 patients.

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.

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Conflict of interest statement

None declared.

References

1. Kudoh S, Fujiwara Y, Takada Y, Yamamoto H, Kinoshita A, Ariyoshi Y, et al. Phase II study of irinotecan combined with cisplatin in patients with previously untreated small-cell lung cancer. West Japan Lung Cancer Group. *J Clin Oncol* 1998;16:1068–74.
2. Negoro S, Fukuoka M, Masuda N, Takada M, Kusunoki Y, Matsui K, et al. Phase I study of weekly intravenous infusions of CPT-11, a new derivative of camptothecin, in the treatment of advanced non-small-cell lung cancer. *J Natl Cancer Inst* 1991;83:1164–8.
3. Rougier P, Van Cutsem E, Bajetta E, Niederle N, Possinger K, Labianca R, et al. Randomised trial of irinotecan versus fluorouracil by continuous infusion after fluorouracil failure in patients with metastatic colorectal cancer. *Lancet* 1998;352:1407–12.
4. Douillard JY, Cunningham D, Roth AD, Navarro M, James RD, Karasek P, et al. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. *Lancet* 2000;355:1041–7.
5. Tournigand C, Andre T, Achille E, Lledo G, Flesh M, Mery-Mignard D, et al. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol* 2004;22:229–37.
6. Ando Y, Saka H, Ando M, Sawa T, Muro K, Ueoka H, et al. Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. *Cancer Res* 2000;60:6921–6.
7. Innocenti F, Undevia SD, Iyer L, Chen PX, Das S, Kocherginsky M, et al. Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. *J Clin Oncol* 2004;22:1382–8.
8. Fujita K, Sasaki Y. Pharmacogenomics in drug-metabolizing enzymes catalyzing anticancer drugs for personalized cancer chemotherapy. *Curr Drug Metab* 2007;8:554–62.
9. Gagne JF, Montminy V, Belanger P, Journault K, Gaucher G, Guillemette C. Common human UGT1A polymorphisms and the altered metabolism of irinotecan active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38). *Mol Pharmacol* 2002;62:608–17.
10. Premawardhena A, Fisher CA, Liu YT, Verma IC, de Silva S, Arambepola M, et al. The global distribution of length polymorphisms of the promoters of the glucuronosyltransferase 1 gene (UGT1A1): hematologic and evolutionary implications. *Blood Cells Mol Dis* 2003;31:98–101.
11. Araki K, Fujita K, Ando Y, Nagashima F, Yamamoto W, Endo H, et al. Pharmacogenetic impact of polymorphisms in the coding region of the UGT1A1 gene on SN-38 glucuronidation in Japanese patients with cancer. *Cancer Sci* 2006;97:1255–9.
12. Han JY, Lim HS, Shin ES, Yoo YK, Park YH, Lee JE, et al. Comprehensive analysis of UGT1A polymorphisms predictive for pharmacokinetics and treatment outcome in patients with non-small-cell lung cancer treated with irinotecan and cisplatin. *J Clin Oncol* 2006;24:2237–44.
13. Minami H, Sai K, Saeki M, Saito Y, Ozawa S, Suzuki K, et al. Irinotecan pharmacokinetics/pharmacodynamics and UGT1A genetic polymorphisms in Japanese: roles of UGT1A1*6 and *28. *Pharmacogenet Genomics* 2007;17:497–504.
14. Fujita K, Ando Y, Nagashima F, Yamamoto W, Eodo H, Araki K, et al. Genetic linkage of UGT1A7 and UGT1A9 polymorphisms to UGT1A1*6 is associated with reduced activity for SN-38 in Japanese patients with cancer. *Cancer Chemother Pharmacol* 2007;60:515–22.
15. Colucci G, Gebbia V, Paoletti G, Giuliani F, Caruso M, Gebbia N, et al. Phase III randomized trial of FOLFIRI versus FOLFOX4 in the treatment of advanced colorectal cancer: a multicenter study of the Gruppo Oncologico Dell'Italia Meridionale. *J Clin Oncol* 2005;23:4866–75.
16. Yasui H, Yoshino T, Boku N, Onozawa Y, Hironaka S, Fukutomi A, et al. Retrospective analysis of S-1 monotherapy in patients with metastatic colorectal cancer after failure to fluoropyrimidine and irinotecan or to fluoropyrimidine, irinotecan and oxaliplatin. *Jpn J Clin Oncol* 2009;39:315–20.
17. Chibaudel B, Maindrault-Goebel F, Lledo G, Mineur L, Andre T, Bennamoun M, et al. Can chemotherapy be discontinued in unresectable metastatic colorectal cancer? The GERCOR OPTIMOX2 Study. *J Clin Oncol* 2009;27:5727–33.
18. Tournigand C, Cervantes A, Figer A, Lledo G, Flesch M, Buyse M, et al. OPTIMOX1: a randomized study of FOLFOX4 or FOLFOX7 with oxaliplatin in a stop-and-Go fashion in advanced colorectal cancer—a GERCOR study. *J Clin Oncol* 2006;24:394–400.
19. Goldberg RM, Sargent DJ, Morton RF, Fuchs CS, Ramanathan RK, Williamson SK, et al. A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. *J Clin Oncol* 2004;22:23–30.
20. Fuchs CS, Marshall J, Mitchell E, Wierzbicki R, Ganju V, Jeffery M, et al. Randomized, controlled trial of irinotecan plus infusional, bolus, or oral fluoropyrimidines in first-line treatment of metastatic colorectal cancer: results from the BICC-C Study. *J Clin Oncol* 2007;25:4779–86.
21. Saltz LB, Clarke S, Diaz-Rubio E, Scheithauer W, Figer A, Wong R, et al. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. *J Clin Oncol* 2008;26:2013–9.
22. Bokemeyer C, Bondarenko I, Makhson A, Hartmann JT, Aparicio J, de Braud F, et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. *J Clin Oncol* 2009;27:663–71.
23. Van Cutsem E, Kohne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009;360:1408–17.

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Sorafenib and Sunitinib, Two Anticancer Drugs, Inhibit CYP3A4-Mediated and Activate CY3A5-Mediated Midazolam 1′-Hydroxylation

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ABSTRACT:

Sorafenib and sunitinib are novel small-molecule molecularly targeted anticancer drugs that inhibit multiple tyrosine kinases. These medicines have shown survival benefits in advanced renal cell carcinomas as well as in advanced hepatocellular carcinomas and gastrointestinal stromal tumors, respectively. The effects of sorafenib and sunitinib on midazolam 1′-hydroxylation catalyzed by human CYP3A4 or CYP3A5 were investigated. Sorafenib and sunitinib inhibited metabolic reactions catalyzed by recombinant CYP3A4. Midazolam hydroxylation was also inhibited in human liver microsomes harboring the *CYP3A5*3/*3* genotype (poor CYP3A5 expressor). In contrast, midazolam 1′-hydroxylation catalyzed by recombinant CYP3A5 was enhanced by the coexistence of sorafenib or sunitinib in a concentration-dependent manner, with saturation occurring at approximately 10 μM. Midazolam hydroxylation was also enhanced in human liver microsomal samples harboring the *CYP3A5*1/*1* genotype (extensive CYP3A5 expressor). Sorafenib N-oxidation and sunitinib N-deethylation, the primary routes of metabolism, were predominantly catalyzed by CYP3A4 but not by CYP3A5. The preincubation period of sorafenib and sunitinib before the midazolam addition in the reaction mixture did not affect the enhancement of CYP3A5-catalyzed midazolam hydroxylation, indicating that the enhancement was caused by parent sorafenib and sunitinib. Docking studies with a CYP3A5 homology model based on the structure of CYP3A4 revealed that midazolam closely docked to the heme of CYP3A5 compared with sorafenib or sunitinib, suggesting that these anticancer drugs act as enhancers, not as substrates. Our results thus showed that sorafenib and sunitinib activated midazolam 1′-hydroxylation by CYP3A5 but inhibited that by CYP3A4. Unexpected drug interactions involving sorafenib and sunitinib might occur via heterotropic cooperativity of CYP3A5.

Introduction

Cytochrome P450 (P450) is a heme-containing enzyme that catalyzes the oxidation of a wide variety of endogenous and exogenous compounds, including therapeutic drugs, carcinogens, and other xenobiotics (Nelson et al., 1996). CYP3A4, one of the major forms, is the most abundant P450 expressed in human livers (Shimada et al., 1994) as well as in the small intestine (Kolars et al., 1992). CYP3A4 has been shown to participate in the metabolism of more than 30% of all therapeutic drugs or 50% of therapeutic drugs that undergo biotransformation (Lamba et al., 2002b; Matsumura et al., 2004). In adults, CYP3A5 is polymorphically expressed in approximately 10 to 40% of whites, 33% of Japanese, and 50% of African Americans (Lamba et al., 2002a). The relative amount of CYP3A5 to total hepatic CYP3A protein varies but can exceed 50% (Kuehl et al., 2001). CYP3A5 shares 84% amino acid sequence homology with CYP3A4. CYP3A5 and CYP3A4 overlap in their substrate specificities, but the relative importance of CYP3A5 and CYP3A4 in overall CYP3A-mediated metabolism differs between substrates (Lamba et al., 2002a; Niwa et al., 2008).

Recent progress in the development of molecularly targeted anticancer drugs has led to the approval of a variety of small-molecule tyrosine kinase inhibitors. These are orally bioavailable molecules that generally reversibly bind to intracellular ATP-binding sites of receptor(s) located inside the cell surface or to binding sites of cytoplasmic factors involved in signal transduction, thereby affecting cell proliferation, apoptosis, or angiogenesis. Sorafenib and sunitinib [chemical

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ABBREVIATIONS: P450, cytochrome P450; FDA, U.S. Food and Drug Administration; HLM, human liver microsomes; HPLC, high-performance liquid chromatography.

structures of sorafenib are found in Wilhelm et al. (2004) and sunitinib in Chow and Eckhardt (2007)] are multikinase inhibitors that target various factors, such as Raf; vascular endothelial growth factor receptors 1, 2, and 3, platelet-derived growth factor receptors α and β, FMS-like tyrosine kinase 3, c-Kit protein, and RET receptor, affecting tumor cell proliferation and tumor angiogenesis (Wilhelm et al., 2004; Carlomagno et al., 2006; Chow and Eckhardt, 2007; Escudier et al., 2007; Llovet et al., 2008). Sorafenib is approved by the U.S. Food and Drug Administration (FDA) and other global health authorities for the treatment of advanced renal and hepatocellular carcinomas, because significant survival advantages were confirmed (Escudier et al., 2007; Llovet et al., 2008). Definitive efficacy of sunitinib has been demonstrated in advanced renal cell carcinoma and in imatinib-refractory gastrointestinal stromal tumors, leading to the FDA approval of sunitinib for the treatment of these diseases (Chow and Eckhardt, 2007). These multikinase inhibitors are predominantly metabolized by CYP3A4. Sorafenib is known to undergo N-oxidation by CYP3A4 (Lathia et al., 2006). Sunitinib N-deethylation, which produces a pharmacologically active metabolite, is also catalyzed by this drug-metabolizing enzyme (Rock et al., 2007). However, the roles of CYP3A5 in the metabolism of these anticancer drugs have not been reported.

Drug-drug interactions have received increasing attention over the past few decades. A recent survey indicated that approximately 30% of the U.S. population older than 57 years takes at least five prescription drugs at any given time (Qato et al., 2008). Many drug-drug interactions involve inhibition of drug-metabolizing enzymes and transporters, resulting in increased systemic exposure and subsequent adverse drug reactions (Zhang et al., 2010). Therefore, evaluation of drug-drug interaction potential is an essential part of risk assessment to ensure the safe use of medicines, including sorafenib and sunitinib. Although information on the effects of CYP3A inhibitors on the metabolism of sorafenib or sunitinib is available (Lathia et al., 2006) (sorafenib prescribing information by the FDA, http://www.accessdata.fda.gov/drugsatfda_docs/label/2007/021923s004s005s006s007lbl.pdf, and sunitinib prescribing information by the FDA, http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021938s010s011s014s015lbl.pdf), the potency of sorafenib or sunitinib for modulating CYP3A4- and CYP3A5-mediated metabolism of other therapeutic drugs has not been reported.

We thus compared the effects of sorafenib and sunitinib on midazolam 1′-hydroxylation, which is catalyzed by both CYP3A4 and CYP3A5. We also examined the contributions of CYP3A5 to sorafenib N-oxidation and sunitinib N-deethylation.

Materials and Methods

Chemicals. Sorafenib, sorafenib *N*-oxide, sunitinib, and *N*-desethylsunitinib were obtained from Toronto Research Chemicals (North York, ON, Canada). Ranitidine hydrochloride and tolnaftate were products of Sigma-Aldrich Japan (Tokyo, Japan). Midazolam hydrochloride and 1′-hydroxymidazolam were from Daiichi Pure Chemicals (Tokyo, Japan). Clonazepam was from Wako Pure Chemicals (Osaka, Japan). All chemicals and solvents were of the highest grade commercially available.

Recombinant Human CYP3A4 and CYP3A5 and Human Liver Microsomes. Recombinant CYP3A4 and CYP3A5 coexpressed with NADPH-P450 reductase and cytochrome b_5 in the microsomes of insect cells with baculovirus systems were purchased from BD Biosciences (Woburn, MA). Insect microsomes were diluted in 100 mM potassium phosphate (pH 7.4). P450 contents were 1000 pmol/ml in both insect microsomal preparations.

Human liver microsomes (HLM) prepared from an African-American man (56 years) possessing *CYP3A5*1/*1* and HLM from a white woman (54 years) harboring *CYP3A5*3/*3* were obtained from BD Biosciences. Testosterone 6β-hydroxylase activities of the *CYP3A5*1/*1* and *CYP3A5*3/*3* HLM as measured by BD Biosciences were 5.8 and 13.6 nmol/min/mg protein, respectively. Expression levels of CYP3A4 and CYP3A5 as determined by Western blotting in the *CYP3A5*1/*1* and *CYP3A5*3/*3* HLM were 170 and 13 and 120 pmol/mg protein and under the detection limit, respectively. All HLM were diluted in 250 mM sucrose. The microsomal protein content was 20 mg/ml.

Effects of Sorafenib and Sunitinib on Midazolam 1′-Hydroxylation by CYP3A4 or CYP3A5. Midazolam 1′-hydroxylation catalyzed by recombinant CYP3A4 or CYP3A5 or by HLM harboring *CYP3A5*1/*1* or **3/*3* was tested as described elsewhere (Fujita et al., 2005). The metabolite 1′-hydroxymidazolam was quantified by the methods of Fujita et al. (2003). Effects of sorafenib and sunitinib on midazolam 1′-hydroxylation were examined as described by Fujita et al. (2005). To determine the inhibition constant (K_i) of sorafenib or sunitinib for midazolam 1′-hydroxylation by recombinant CYP3A4, concentrations of sorafenib and sunitinib ranged from 1 to 10 and 1 to 20 μM, respectively. Concentrations of midazolam ranging from 5 to 20 μM were used to estimate the K_i. K_i values were calculated by nonlinear regression analysis, using eq. 1 for competitive inhibition with GraphPad Prism (version 5; GraphPad Software, Inc., San Diego, CA):

$$v = V_{max} \cdot [S]/(K_m \cdot (1+[I]/K_i)+[S]) \quad (1)$$

where v, V_{max}, $[S]$, K_m, and $[I]$ are velocity, maximum velocity, substrate concentration, Michaelis constant, and inhibitor concentration, respectively. K_i is reported herein as the mean ± S.E.

Sorafenib N-Oxidation by CYP3A4 or CYP3A5. Sorafenib N-oxidation by recombinant CYP3A4 or CYP3A5 was examined as follows. A typical incubation mixture consisted of 100 mM sodium potassium phosphate buffer (pH 7.4), an NADPH-generating system (1.3 mM $NADP^+$, 3.3 mM $MgCl_2$, 3.8 mM glucose 6-phosphate, and 0.4 unit/ml glucose-6-phosphate dehydrogenase), and recombinant CYP3A4 or CYP3A5 in a final volume of 0.25 ml. The time-dependent formation of sorafenib *N*-oxide was assessed with a sorafenib concentration of 50 μM and a P450 concentration of 20 nM. Sorafenib *N*-oxide was analyzed by a computerized model 7000 HPLC system (Hitachi, Tokyo, Japan) as described previously (Afify et al., 2004) with slight modifications. In brief, the HPLC system was equipped with a TSKgel ODS-120T analytical column (4.6 × 250 mm, 4 μm; TOSOH, Tokyo, Japan). The metabolite was quantified by comparing the HPLC peak area to that of the internal standard tolnaftate. The lower limit of quantification of sorafenib *N*-oxide was 0.06 μM. The intra- and interassay coefficients of variation at 2 μM sorafenib *N*-oxide were less than 4.3 and 4.5%, respectively. Each assay was performed three times in duplicate.

Sunitinib N-Deethylation by CYP3A5 or CYP3A4. Sunitinib N-deethylation by recombinant CYP3A4 or CYP3A5 was examined using the same methods as described for sorafenib N-oxidation. The time-dependent formation of *N*-desethylsunitinib was assessed at a sunitinib concentration of 200 μM and a P450 concentration of 20 nM. *N*-Desethylsunitinib was analyzed by a computerized HPLC system (model 7000 series) as described previously (Blanchet et al., 2009) with minor modifications. In brief, the HPLC system was equipped with a YMC-Pack CN analytical column (4.6 × 250 mm, 5 μm; YMC, Kyoto, Japan). The metabolite was quantified by comparing the HPLC peak area with that of the internal standard ranitidine. The lower limit of quantification of *N*-desethylsunitinib was 0.25 μM. The intra- and interassay coefficients of variation at 0.8 μM *N*-desethylsunitinib were less than 7.9 and 7.2%, respectively. Each assay was performed three times in duplicate.

Enzyme Kinetics of Sorafenib N-Oxidation and Sunitinib N-Deethylation. In the assays of sorafenib N-oxidation by recombinant CYP3A4, the P450 content and reaction time were predetermined with 50 μM sorafenib, on the basis of the linearity between the microsomal P450 concentration (up to 20 nM) and the reaction time (up to 5 min) versus the rate of metabolite formation. On the basis of the results, the P450 content and the reaction time were determined to be 20 nM and 5 min, respectively. The concentrations of sorafenib for enzyme kinetics ranged from 1.9 to 30 μM for CYP3A4. In the assays of sunitinib *N*-deethylation, the protein content and the reaction time were determined to be 20 nM and 10 min, respectively, for CYP3A4-expressing microsomes and 20 nM and 20 min, respectively, for CYP3A5-expressing microsomes. The concentrations of sunitinib for enzyme kinetics ranged from 12.5 to 200 μM for CYP3A4 and from 18.8 to 300 μM for CYP3A5. Data points were fitted to the Michaelis-Menten equation by nonlinear least-squares

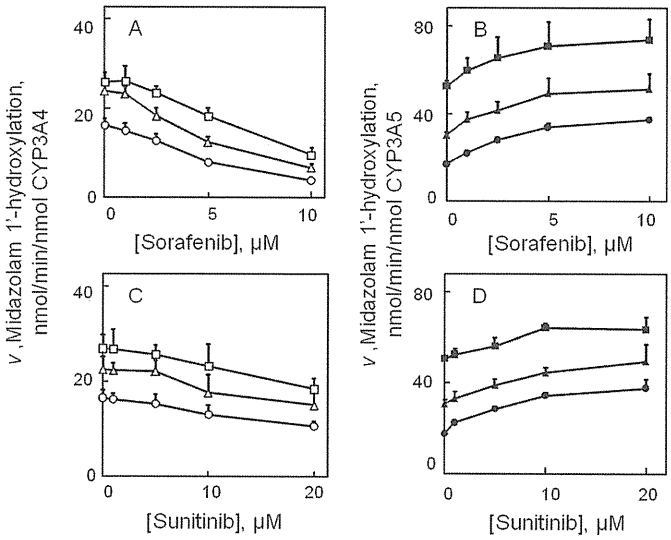

FIG. 1. Modulation of midazolam 1′-hydroxylation activities catalyzed by CYP3A4 (A and C, open symbols) or CYP3A5 (B and D, filled symbols) in the presence of sorafenib (A and B) or sunitinib (C and D). Midazolam 1′-hydroxylation activities were determined at substrate concentrations of 5 (○, ●), 10 (△, ▲), and 20 (□, ■) μM, respectively. Each point shows the mean of three independent analyses with S.D.

regression analysis with the use of GraphPad Prism software. Kinetic constants (K_m and V_{max}) are reported as the means ± S.E.

Docking Simulation of Sorafenib or Sunitinib into the Reported Structure of CYP3A4 and a Homology Model of CYP3A5. Docking simulation was performed as described elsewhere (Okada et al., 2009; Shimada et al., 2010). The CYP3A5 primary sequence was aligned with CYP3A4 (Protein Data Bank code 1TQN) in MOE software (version 2007.09; Chemical Computing Group, Montreal, QC, Canada) to model a three-dimensional structure (Pearson et al., 2007). Before docking, the energy of the CYP3A4 or CYP3A5 structure was minimized by using the CHARM22 force field. Docking simulations were performed for sorafenib or sunitinib binding to the reported CYP3A4 or a homology model of CYP3A5 in the presence or absence of midazolam, using the MMFF94x force field distributed in the MOE Dock software. Twenty solutions were generated for each docking experiment and were ranked according to total interaction energy (U value).

Results

Modulation of Midazolam 1′-Hydroxylation by CYP3A4 or CYP3A5 with Sorafenib or Sunitinib. As shown in Fig. 1, A and C, sorafenib and sunitinib inhibited midazolam 1′-hydroxylation catalyzed by CYP3A4 in a dose-dependent manner. We calculated the K_i values of sorafenib and sunitinib for midazolam 1′-hydroxylation by CYP3A4. The K_i values of sorafenib and sunitinib were estimated to be 1.7 ± 0.3 and 12 ± 0.9 μM, respectively. The inhibition of midazolam 1′-hydroxylation by sorafenib or sunitinib was also observed with CYP3A5-deficient HLM (*CYP3A5*3/*3*) (Fig. 2A). In contrast, midazolam 1′-hydroxylation by CYP3A5 was enhanced by the coexistence of sorafenib or sunitinib in the reaction mixture (Fig. 1, B and D). The enhancement was dependent on the concentration of sorafenib or sunitinib, with saturation occurring at a concentration of approximately 10 μM. Similar enhancement was observed with HLM (*CYP3A5*1/*1*) expressing CYP3A5 (Fig. 2B). α-Naphthoflavone was used as a positive control for the enhancement of CYP3A activity, because this chemical has been well known to show heterotropic positive cooperativity for various metabolic reactions catalyzed by CYP3A (Hutzler and Tracy, 2002). As expected, α-naphthoflavone activated the midazolam 1′-hydroxylation by HLM genotyped for the *CYP3A5* gene.

Contributions of CYP3A5 to Sorafenib N-Oxidation or Sunitinib N-Deethylation. To address whether or not the enhancement of CYP3A5-catalyzed midazolam 1′-hydroxylation was attributed to parent sorafenib or sunitinib, we next examined the roles of CYP3A5 and CYP3A4 in sorafenib N-oxidation and sunitinib N-deethylation. The time courses of sorafenib N-oxidation by CYP3A4 or CYP3A5 are shown in Fig. 3A. Sorafenib N-oxidation by CYP3A4 was 17 times higher than that by CYP3A5 at 20 min of incubation (190 versus 11 nmol/nmol P450). The kinetics of CYP3A4-mediated sorafenib metabolism were determined (Fig. 3C). We could not determine the kinetics of CYP3A5-mediated sorafenib metabolism, because the velocity of the reaction was not saturated by a concentration of 90 μM. Sorafenib was not soluble in the solvent used in the present study (1% dimethyl sulfoxide) at concentrations higher than 90 μM. The apparent K_m and V_{max} values of sorafenib *N*-oxidation by CYP3A4 were 6.1 ± 0.7 μM and 18 ± 0.7 nmol/min/nmol CYP3A4, respectively. The intrinsic metabolic clearance calculated with the K_m and V_{max} values obtained for CYP3A4 was 3.0 μl/min/pmol CYP3A4.

The time courses of sunitinib N-deethylation examined with CYP3A4 or CYP3A5 are shown in Fig. 3B. Sunitinib N-deethylation was 3.0 times higher with CYP3A4 than with CYP3A5 at 20 min of incubation (1100 versus 370 nmol/nmol CYP3A). The kinetics of CYP3A4- or CYP3A5-mediated sunitinib metabolism were estimated (Fig. 3D). The apparent K_m and V_{max} values of sunitinib N-deethylation by CYP3A4 and CYP3A5 were 32 ± 3.3 and 110 ± 12 μM and 97 ± 3.3 and 35 ± 1.7 nmol/min/nmol CYP3A, respectively. The intrinsic metabolic clearance calculated with the K_m and V_{max} values obtained with CYP3A4 was 10 times higher than that obtained with CYP3A5 (3.0 versus 0.3 μl/min/pmol CYP3A).

Thus, we found that sorafenib and sunitinib are substrates for CYP3A4 but not for CYP3A5. The parent compounds sorafenib and sunitinib are activators of CYP3A5-mediated midazolam 1′-hydroxylation.

Effects of Preincubation of Sorafenib or Sunitinib on CYP3A5-Catalyzed Midazolam 1′-Hydroxylation. To further test whether or

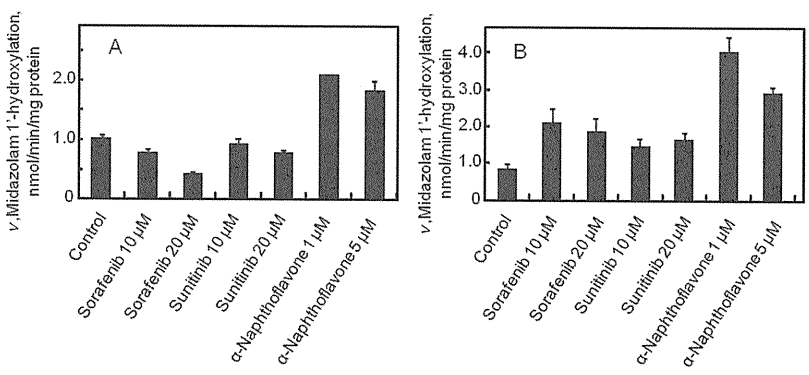

FIG. 2. Effects of sorafenib and sunitinib on midazolam 1′-hydroxylation by human liver microsomes genotyped for *CYP3A5*3/*3* (A) or *CYP3A5*1/*1* (B). The midazolam concentration used was 10 μM. Each data point shows the mean of triplicate determinations with S.D. bars.

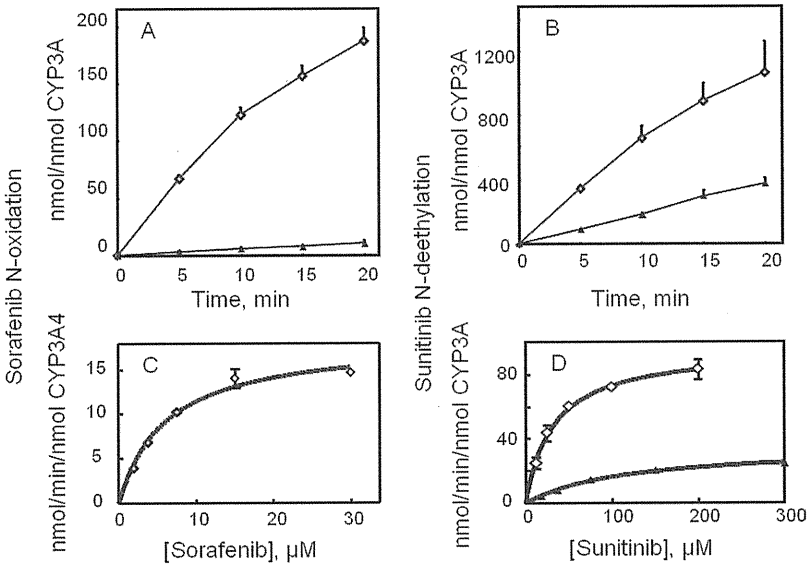

FIG. 3. Sorafenib N-oxidation and sunitinib N-deethylation activities catalyzed by CYP3A4 (A and C) or CYP3A5 (B and D). Time courses of sorafenib N-oxidation (A) and sunitinib N-deethylation (B) by CYP3A4 (◇) or CYP3A5 (▲) and effects of substrate concentrations on sorafenib N-oxidation (C) and sunitinib N-deethylation (D) activities by CYP3A4 (◇) or CYP3A5 (▲) are shown. Each data point represents the mean of triplicate determinations and is shown with S.D. bars.

not enhancement of midazolam 1′-hydroxylation by recombinant CYP3A5 was caused by parent sorafenib or sunitinib, effects of the elongation of preincubation of sorafenib or sunitinib with NADPH on midazolam 1′-hydroxylation by CYP3A5 were examined. As expected, enhancement of midazolam 1′-hydroxylation was not affected by the elongation of preincubation period (Fig. 4).

Docking Simulation of Sorafenib or Sunitinib into CYP3A4 and CYP3A5. The human CYP3A4 crystal structure allowed generation of a homology model of CYP3A5, derived with the MOE program. The top-rank docking model of sorafenib or sunitinib with or without midazolam in CYP3A4 and CYP3A5 was used. Docking simulation of sorafenib or sunitinib into CYP3A4 and CYP3A5 was first performed without midazolam. Moieties in sorafenib and sunitinib, which are metabolized by CYP3A4, closely docked to the heme of CYP3A4 (Fig. 5, A and C) with low U energy values (−36.0 and −42.6). In contrast, these moieties were found far from the heme of CYP3A5 (−25.6 and −37.2, respectively) (Fig. 5, B and D).

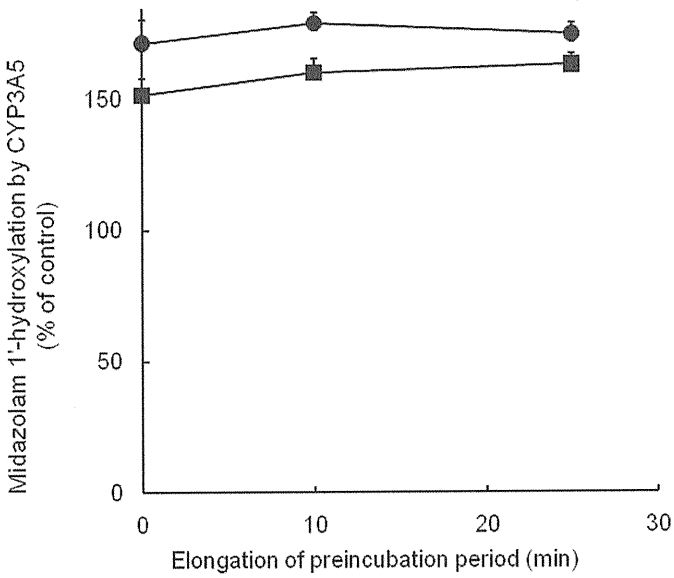

FIG. 4. Effects of the preincubation period with 10 μM sorafenib (●) or 20 μM sunitinib (■) in the presence of NADPH on recombinant CYP3A5-mediated midazolam hydroxylation activities at a substrate concentration of 10 μM.

In the presence of midazolam, sorafenib or sunitinib molecules were also located at a similar distance from the heme of CYP3A4 (Fig. 6, A and C) with relatively high U energy values. In contrast, midazolam closely docked to the heme of CYP3A5 even in the presence of sorafenib or sunitinib (Fig. 6, B and D).

Discussion

The present study demonstrated that sorafenib and sunitinib inhibited midazolam 1′-hydroxylation catalyzed by CYP3A4 but enhanced that catalyzed by CYP3A5 (Figs. 1 and 2). The enhancement of midazolam 1′-hydroxylation by CYP3A5 was most likely caused by parent sorafenib and sunitinib, not by their metabolites, because these small-molecule tyrosine kinase inhibitors were only slightly metabolized by this drug-metabolizing enzyme (Fig. 3). In addition, the enhancement of midazolam 1′-hydroxylation was not affected by the preincubation period of either sorafenib or sunitinib with NADPH and CYP3A5 (Fig. 4). This result further supports the notion that the enhancement of midazolam 1′-hydroxylation by CYP3A5 was induced by parent sorafenib or sunitinib.

The enhancement of metabolic reactions in the presence of modulator(s), a phenomenon referred to as heterotropic cooperativity, involves two different ligands in the active site of a P450 enzyme (Hutzler and Tracy, 2002; Isin and Guengerich, 2006). Heterotropic cooperativity in CYP3A5 was recently reported by Okada et al. (2009). In their study, thalidomide greatly enhanced midazolam 1′-hydroxylation and cyclosporine oxidation by CYP3A5, consistent with our findings. These results suggest that a typical ligand universally induces heterotropic cooperativity in CYP3A5-mediated metabolism. Of interest, however, in our other experiments using erlotinib, a small-molecule tyrosine kinase inhibitor of epidermal growth factor receptor, as a substrate of CYP3A4 and CYP3A5 (Li et al., 2007), both sorafenib and sunitinib inhibited erlotinib O-demethylation (data not shown). Our results suggest that the heterotropic cooperativity in CYP3A5-mediated metabolism associated with sorafenib or sunitinib may depend on metabolic reactions catalyzed by CYP3A5. Further studies are needed to confirm this point.

Mechanisms underlying the modulation of CYP3A4- and CYP3A5-mediated midazolam 1′-hydroxylation by sorafenib or sunitinib may be partly explained by the results of docking simulation. As shown in Fig. 6, A and C, sorafenib or sunitinib seemed to interfere with the binding of midazolam to the heme of

CYP3A4, because these anticancer drugs and midazolam were located at similar distances from the heme of CYP3A4. These results suggest that sorafenib or sunitinib competitively inhibit CYP3A4-catalyzed midazolam 1′-hydroxylation.

In contrast, midazolam preferentially docked closely to the heme of CYP3A5, even in the coexistence of sorafenib or sunitinib (Fig. 6, B and D). Therefore, sorafenib or sunitinib appears not to inhibit CYP3A5-mediated midazolam 1′-hydroxylation. These anticancer drugs located far from the heme may induce heterotropic cooperativity in CYP3A5-mediated metabolism, although the detailed mechanisms remain unclear.

We found that sorafenib and sunitinib were extensively metabolized by CYP3A4 but not by CYP3A5 (Fig. 3). The docking model showed that the functional groups in sorafenib and sunitinib that are metabolized by CYP3A4 docked close to the heme of the enzyme but were located far from the heme of CYP3A5 (Fig. 5). These results supported the observations that sorafenib and sunitinib were good substrates of CYP3A4 but not of CYP3A5.

The roles of CYP3A4 and CYP3A5 in sorafenib and sunitinib metabolism were examined with HLM (*CYP3A5*1/*1*) expressing CYP3A5 and CYP3A5-deficient HLM (*CYP3A5*3/*3*), respectively. Sorafenib N-oxidation by *CYP3A5*1/*1* and *CYP3A5*3/*3* HLM was 0.32 ± 0.010 and 0.72 ± 0.021 pmol/min/mg protein, respectively (n = 3). Sunitinib N-deethylation by *CYP3A5*1/*1* and *CYP3A5*3/*3* HLM was 1.6 ± 0.027 and 4.9 ± 0.16 pmol/min/mg protein, respectively (n = 3). Respective sorafenib and sunitinib concentrations were 10 and 100 μM, and the incubation period was 10 min. These results suggest that the contribution of CYP3A5 to the metabolism of sorafenib and sunitinib might be low, which supports the results obtained with recombinant CYP3A4 and CYP3A5. However, it might be difficult to quantitatively evaluate the contributions of CYP3A4 and CYP3A5 to the metabolism of sorafenib and sunitinib because of reasons such as different levels of CYP3A4 expression in these microsomal preparations. Therefore, we examined the roles of CYP3A4 and CYP3A5 in the sorafenib N-oxidation and sunitinib N-deethylation with recombinant CYP3A4 and CYP3A5.

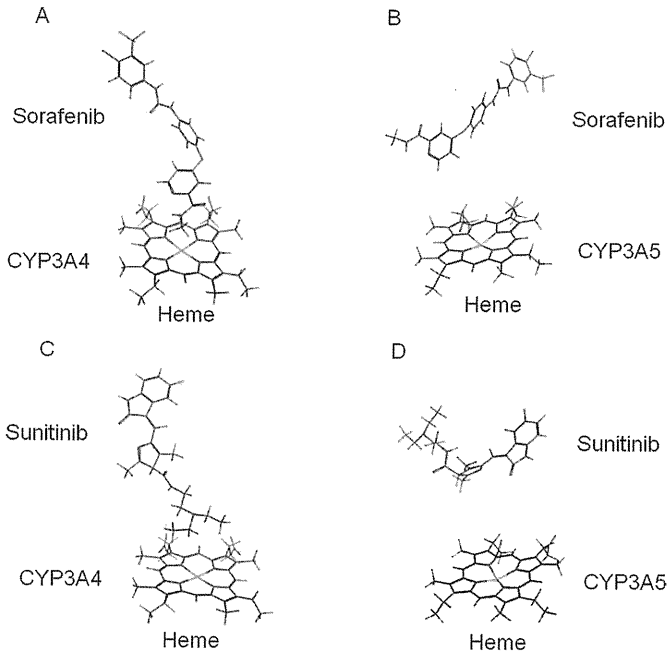

FIG. 5. Docking simulation of sorafenib (A and B) or sunitinib (C and D) into CYP3A4 (A and C) and CYP3A5 (B and D) in the absence of midazolam. Drug-P450 interaction energies (U, kilocalories per mole), calculated in Fig. 5, A to D, were −36.0, −25.6, −42.6, and −37.2, respectively. In the figure, oxygen, nitrogen, fluoride, and iron atoms are colored with red, blue, green, and light blue, respectively.

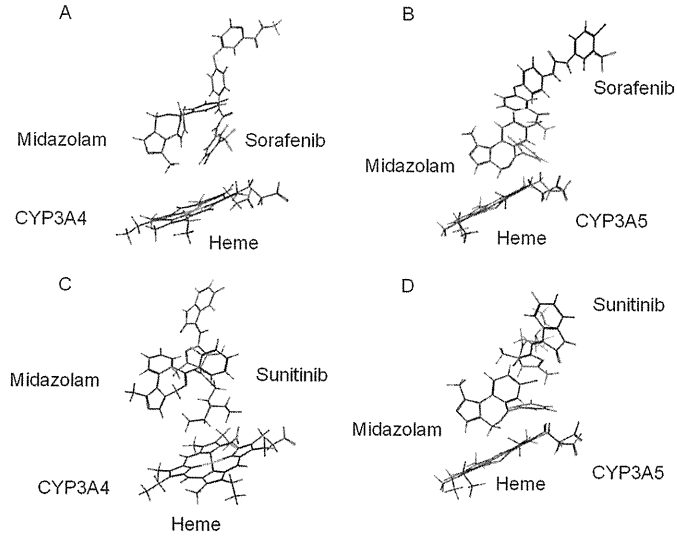

FIG. 6. Docking simulation of sorafenib (A and B) or sunitinib (C and D) into CYP3A4 (A and C) and CYP3A5 (B and D) in the presence of midazolam. Drug-P450 interaction energies (U, kilocalories per mole), calculated in Fig. 6, A to D, were 77.2, 259, 276, and 360, respectively. Other details were as in the legend to Fig. 5.

When 400 mg of sorafenib was given twice daily, the plasma concentration of sorafenib measured after the 14 days reached approximately 9 μM (maximum and trough concentrations 10 and 8 μM, respectively) (Minami et al., 2008). Because the activation of CYP3A5-mediated midazolam 1′-hydroxylation was observed at a sorafenib concentration of 9 μM (Fig. 1B), this medicine may activate the CYP3A5-mediated metabolism in clinical practice. In contrast, sunitinib might not affect the midazolam 1′-hydroxylation in clinical use, because the maximum plasma concentration assayed 28 days after the sunitinib treatment (50 mg/daily) was approximately 0.17 μM (Shirao et al., 2010).

Considerable interpatient variability exists in the pharmacokinetics of sorafenib (Minami et al., 2008). Our present results suggest that individual variability in the pharmacokinetics of sorafenib might be substantially associated with variable expression levels of CYP3A4, differing by more than 40 times in the liver and the small intestine, but might not be linked to genetic polymorphisms seen in CYP3A5 (Lamba et al., 2002a). In support of our hypothesis, the *CYP3A5*3* allele, which is associated with no or poor expression of functional CYP3A5 (Kuehl et al., 2001), was not significantly related to interindividual variability in the pharmacokinetics of sorafenib (Lind et al., 2010).

In conclusion, we found that sorafenib and sunitinib inhibited midazolam 1′-hydroxylation by CYP3A4 but enhanced that by CYP3A5. The present study suggests that midazolam metabolism may be increased by sorafenib and sunitinib through the heterotropic cooperativity of human CYP3A5. Because of the high frequency of polymorphic CYP3A5 expression in Asians and Africans, a relatively high frequency of unexpected drug interactions involving sorafenib (and sunitinib) might occur via CYP3A5 contribution in drug metabolism.

Authorship Contributions

Participated in research design: Sugiyama and Fujita.
Conducted experiments: Sugiyama and Akiyama.
Performed data analysis: Sugiyama and Fujita.

Wrote or contributed to the writing of the manuscript: Sugiyama, Fujita, and Yamazaki.

Other: Murayama and Yamazaki performed docking simulation of sorafenib and sunitinib, and Sasaki acquired funding for the research.

References

Afify S, Rapp UR, and Hogger P (2004) Validation of a liquid chromatography assay for the quantification of the Raf kinase inhibitor BAY 43-9006 in small volumes of mouse serum. *J Chromatogr B Analyt Technol Biomed Life Sci* **809:**99–103.

Blanchet B, Saboureau C, Benichou AS, Billemont B, Taieb F, Ropert S, Dauphin A, Goldwasser F, and Tod M (2009) Development and validation of an HPLC-UV-visible method for sunitinib quantification in human plasma. *Clin Chim Acta* **404:**134–139.

Carlomagno F, Anaganti S, Guida T, Salvatore G, Troncone G, Wilhelm SM, and Santoro M (2006) BAY 43-9006 inhibition of oncogenic RET mutants. *J Natl Cancer Inst* **98:**326–334.

Chow LQ and Eckhardt SG (2007) Sunitinib: from rational design to clinical efficacy. *J Clin Oncol* **25:**884–896.

Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, Negrier S, Chevreau C, Solska E, Desai AA, et al. (2007) Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med* **356:**125–134.

Fujita K, Ando Y, Narabayashi M, Miya T, Nagashima F, Yamamoto W, Kodama K, Araki K, Endo H, and Sasaki Y (2005) Gefitinib (Iressa) inhibits the CYP3A4-mediated formation of 7-ethyl-10-(4-amino-1-piperidino)carbonyloxycamptothecin but activates that of 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin from irinotecan. *Drug Metab Dispos* **33:**1785–1790.

Fujita K, Hidaka M, Takamura N, Yamasaki K, Iwakiri T, Okumura M, Kodama H, Yamaguchi M, Ikenoue T, and Arimori K (2003) Inhibitory effects of citrus fruits on cytochrome P450 3A (CYP3A) activity in humans. *Biol Pharm Bull* **26:**1371–1373.

Hutzler JM and Tracy TS (2002) Atypical kinetic profiles in drug metabolism reactions. *Drug Metab Dispos* **30:**355–362.

Isin EM and Guengerich FP (2006) Kinetics and thermodynamics of ligand binding by cytochrome P450 3A4. *J Biol Chem* **281:**9127–9136.

Kolars JC, Schmiedlin-Ren P, Schuetz JD, Fang C, and Watkins PB (1992) Identification of rifampin-inducible P450IIIA4 (CYP3A4) in human small bowel enterocytes. *J Clin Invest* **90:**1871–1878.

Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, et al. (2001) Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* **27:**383–391.

Lamba JK, Lin YS, Schuetz EG, and Thummel KE (2002a) Genetic contribution to variable human CYP3A-mediated metabolism. *Adv Drug Deliv Rev* **54:**1271–1294.

Lamba JK, Lin YS, Thummel K, Daly A, Watkins PB, Strom S, Zhang J, and Schuetz EG (2002b) Common allelic variants of cytochrome P4503A4 and their prevalence in different populations. *Pharmacogenetics* **12:**121–132.

Lathia C, Lettieri J, Cihon F, Gallentine M, Radtke M, and Sundaresan P (2006) Lack of effect of ketoconazole-mediated CYP3A inhibition on sorafenib clinical pharmacokinetics. *Cancer Chemother Pharmacol* **57:**685–692.

Li J, Zhao M, He P, Hidalgo M, and Baker SD (2007) Differential metabolism of gefitinib and erlotinib by human cytochrome P450 enzymes. *Clin Cancer Res* **13:**3731–3737.

Lind JS, Dingemans AM, Groen HJ, Thunnissen FB, Bekers O, Heideman DA, Honeywell RJ, Giovannetti E, Peters GJ, Postmus PE, et al. (2010) A multicenter phase II study of erlotinib and sorafenib in chemotherapy-naive patients with advanced non-small cell lung cancer. *Clin Cancer Res* **16:**3078–3087.

Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, et al. (2008) Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* **359:**378–390.

Matsumura K, Saito T, Takahashi Y, Ozeki T, Kiyotani K, Fujieda M, Yamazaki H, Kunitoh H, and Kamataki T (2004) Identification of a novel polymorphic enhancer of the human CYP3A4 gene. *Mol Pharmacol* **65:**326–334.

Minami H, Kawada K, Ebi H, Kitagawa K, Kim YI, Araki K, Mukai H, Tahara M, Nakajima H, and Nakajima K (2008) Phase I and pharmacokinetic study of sorafenib, an oral multikinase inhibitor, in Japanese patients with advanced refractory solid tumors. *Cancer Sci* **99:**1492–1498.

Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, Waterman MR, Gotoh O, Coon MJ, Estabrook RW, et al. (1996) P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* **6:**1–42.

Niwa T, Murayama N, Emoto C, and Yamazaki H (2008) Comparison of kinetic parameters for drug oxidation rates and substrate inhibition potential mediated by cytochrome P450 3A4 and 3A5. *Curr Drug Metab* **9:**20–33.

Okada Y, Murayama N, Yanagida C, Shimizu M, Guengerich FP, and Yamazaki H (2009) Drug interactions of thalidomide with midazolam and cyclosporine A: heterotropic cooperativity of human cytochrome P450 3A5. *Drug Metab Dispos* **37:**18–23.

Pearson JT, Wahlstrom JL, Dickmann LJ, Kumar S, Halpert JR, Wienkers LC, Foti RS, and Rock DA (2007) Differential time-dependent inactivation of P450 3A4 and P450 3A5 by raloxifene: a key role for C239 in quenching reactive intermediates. *Chem Res Toxicol* **20:**1778–1786.

Qato DM, Alexander GC, Conti RM, Johnson M, Schumm P, and Lindau ST (2008) Use of prescription and over-the-counter medications and dietary supplements among older adults in the United States. *JAMA* **300:**2867–2878.

Rock EP, Goodman V, Jiang JX, Mahjoob K, Verbois SL, Morse D, Dagher R, Justice R, and Pazdur R (2007) Food and Drug Administration drug approval summary: sunitinib malate for the treatment of gastrointestinal stromal tumor and advanced renal cell carcinoma. *Oncologist* **12:**107–113.

Shimada T, Tanaka K, Takenaka S, Murayama N, Martin MV, Foroozesh MK, Yamazaki H, Guengerich FP, and Komori M (2010) Structure-function relationships of inhibition of human cytochromes P450 1A1, 1A2, 1B1, 2C9, and 3A4 by 33 flavonoid derivatives. *Chem Res Toxicol* doi:10.1021/tx100286d.

Shimada T, Yamazaki H, Mimura M, Inui Y, and Guengerich FP (1994) Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* **270:**414–423.

Shirao K, Nishida T, Doi T, Komatsu Y, Muro K, Li Y, Ueda E, and Ohtsu A (2010) Phase I/II study of sunitinib malate in Japanese patients with gastrointestinal stromal tumor after failure of prior treatment with imatinib mesylate. *Invest New Drugs* **28:**866–875.

Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, Chen C, Zhang X, Vincent P, McHugh M, et al. (2004) BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res* **64:**7099–7109.

Zhang L, Reynolds KS, Zhao P, and Huang SM (2010) Drug interactions evaluation: an integrated part of risk assessment of therapeutics. *Toxicol Appl Pharmacol* **243:**134–145.

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＜レポート＞

我が国における「急性肝不全」の概念，診断基準の確立：厚生労働省科学研究費補助金（難治性疾患克服研究事業）「難治性の肝・胆道疾患に関する調査研究」班，ワーキンググループ-1，研究報告

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要旨：我が国の劇症肝炎は肝炎像を伴う症例のみを対象としており，欧米の急性肝不全とは疾患単位が異なる．また，その診断基準にはプロトロンビン時間の％表記を採用しており，INR表記を用いている諸外国とは整合性がとれない．そこで，厚生労働省研究班は，欧米の整合性に配慮した「急性肝不全」の診断基準を作成した．同基準では，正常肝ないし肝機能が正常と考えられる肝に肝障害が生じ，初発症状出現から8週以内に，高度の肝機能障害に基づいてプロトロンビン時間が40％以下ないしはINR値1.5以上を示すものを「急性肝不全」と診断することとした．また，肝性脳症が認められない，ないしは昏睡度がI度までの「非昏睡型」，昏睡II度以上の肝性脳症を呈する「昏睡型」に分類し，後者は初発症状出現から昏睡II度以上の肝性脳症が出現するまでの期間が10日以内の「急性型」と，11日以降56日以内の「亜急性型」に区分することにした．この診断基準に注記を併記し，従来の劇症肝炎との関連および成因分類を明確にした．

索引用語：　急性肝不全　　劇症肝炎　　遅発性肝不全　　肝性脳症

1．はじめに

わが国の急性肝不全は肝炎ウイルス感染に起因する症例が多く[1)]，アセトアミノフェン中毒が主体である欧米のacute liver failureとは，患者背景および臨床経過が異なっている[2)]．このため，我が国における急性肝不全の代表疾患は劇症肝炎であり，その診断は1981年に犬山シンポジウムで作成された基準に準拠している[3)]．同基準では，劇症肝炎を病理組織学的にリンパ球浸潤などの肝炎像を呈する急性肝不全に限定している．このため，劇症肝炎の成因は肝炎ウイルス感染，自己免疫性肝炎，薬物アレルギーに分類され，何れにも該当しない場合は成因不明例として扱ってきた[1)]．従って，薬物中毒，循環障害，術後肝不全，妊娠脂肪肝など肝炎像を呈しない急性肝不全は劇症肝炎から除外しているが，欧米ではこれらも含めてacute liver failureと診断している．厚生労働省「難治性の肝疾患に関する研究」班（研究代表者：戸田剛太郎）は，2002～2003年に犬山シンポジウムの診断基準の見直し作業を行ったが，改変した診断基準も原則的に1981年のものを踏襲している[4)]．

以上のように，我が国の劇症肝炎と欧米のacute liver failureでは疾患単位が異なるが，診断に用いる指標に関しても両者の整合性はとれていない．これはプロトロンビン時間の扱いに関してで，我が国では40％以下を劇症肝炎の基準として採用しているが，欧米ではINR表記が一般化しており，成績を比較するのが困難であ

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る．

これら劇症肝炎の診断に関する問題を解決すべく，厚生労働省科学研究費補助金（難治性疾患克服研究事業)「難治性の肝・胆道疾患に関する調査研究」班（研究代表者：坪田博仁）はワーキンググループを設立し（責任者：持田　智），欧米との整合性を考慮した「急性肝不全」の診断基準を作成した[5)~7)]．

2. 方　法

1）プロトロンビン時間の測定方法に関する検討(担当：中山伸朗)

日本肝臓学会の理事，評議員（地方会も含む）の所属する454施設（550診療科）を対象に，PTの測定に用いているキットのアンケート調査を実施した．アンケートで回答のあったキットに関しては，製造メーカーに連絡し，そのISI値と％表記で40％に相当するINRの数値に関して問い合わせた．

2）我が国における劇症肝炎以外の急性肝不全に関する検討（担当；滝川康裕）

全国に救急センター553施設(救急救命センター218施設および救急科専門医指定の235施設）を対象として，2006～2008年に発症した急性肝不全のアンケート調査を実施した．急性肝不全の基準は「発症から8週以内にPTが50％以下またはINR値が1.5以上を呈する症例，ないしは肝性脳症を発症した症例で，劇症肝炎，LOHFには分類されないもの」とし，「非代償性肝硬変を除く慢性肝疾患の先行している症例」も含めて集計した．登録された症例の病型は，非昏睡型急性肝不全，劇症肝炎急性型に準じる症例，劇症肝炎亜急性型に準じる症例，LOHFに準じる症例，acute-on-chronic型肝不全の5型に分類した．

3）診断基準の作成

劇症肝炎および遅発性肝不全(late onset hepatic failure：LOHF)の全国調査[1)9)~13)]，およびプロトロンビン時間の測定方法に関する検討，我が国における劇症肝炎以外の急性肝不全に関する検討の成績[6)]を基に，ワーキンググループの構成員が，急性肝不全の定義，診断基準に関して討議した．ワーキンググループで作成した「急性肝不全の診断基準」(案）は，研究班の研究分担者，研究協力者に提示し，その意見を基に修正，加筆を行って完成させた．

3. 結　果

1）プロトロンビン時間の測定方法に関する検討

350施設（387診療科）から回答が得られた．これらの施設で利用されているキットは16種類で，トロンボレルS（シスメックス社）が138施設（39％）で最も多く，トロンボプラスチンCプラス(シスメックス社)50施設（14％），トロンボチェックPT（シスメックス社）47施設（13％）が次ぎ，上位6種類のキットで全体の92％を占めていた．これら16種類のキットのISI値は0.81から2.05に，％表記で40％に相当するINR値は1.6から2.12に分布していた．但し，使用頻度で上位6位までのキットでは，40％に相当するINR値は1.6から1.98に分布し，その平均は1,86であった．

2）我が国における劇症肝炎以外の急性肝不全に関する検討

58施設（10％）から217例が登録された．病型別では非昏睡型急性肝不全が79例（36％），劇症肝炎急性型に準じる症例が58例（27％），劇症肝炎亜急性型に準じる症例が34例（16％），LOHFに準じる症例が2例（1％），acute-on-chronic型肝不全が44例（20％）であった．また，成因は劇症肝炎と同様のウイルス性，自己免疫性，薬物性，成因不明例の何れかに相当するのが121例(56％），劇症肝炎には分類されないものが96例（44％）であった．これらの成因で最も多いのはアルコール性（43例）であり，以下は循環障害（18例），悪性腫瘍浸潤（8例），中毒性（8例），術後肝不全（2例），代謝性疾患（1例）の順で，その他は16例であった．これら劇症肝炎以外の症例の成因は，acute-on-chronic型肝不全ないしは非昏睡型急性肝不全が大部分を占めていた．

救命率は非昏睡型急性肝不全が78％と高率であったが，劇症肝炎急性型に準じる症例は35％，劇症肝炎亜急性型に準じる症例は12％，LOHFに準じる症例が0％，acute-on-chronic型肝不全は21％と低率であった．なお，肝移植は劇症肝炎急性型に準じる症例の10％，劇症肝炎亜急性型に準じる症例は12％，LOHFに準じる症例が50％で実施されていた．死因は肝不全以外の合併症が44症例（40％）と多く，特に非昏睡型急性肝不全ではその比率が87％と高率であった．特にアルコール性の症例はacute-on-chronic型肝不全に分類される症例が多く，多臓器不全で死亡する頻度が高率であった．

3）診断基準の作成

我が国における「急性肝不全の診断基準」はTable 1のように決定した．診断基準には従来の劇症肝炎，LOHFとの関連を明確にするための注記を追記し，さらに肝性脳症の程度（Table 2，3）と成因の診断（Table 4）に関する資料を添付した．

Table 1　急性肝不全の診断基準

正常肝ないし肝予備能が正常と考えられる肝に肝障害が生じ，初発症状出現から8週以内に，高度の肝機能障害に基づいてプロトロンビン時間が40%以下ないしはINR値1.5以上を示すものを「急性肝不全」と診断する．急性肝不全は肝性脳症が認められない，ないしは昏睡度がⅠ度までの「非昏睡型」と，昏睡Ⅱ度以上の肝性脳症を呈する「昏睡型」に分類する．また，「昏睡型急性肝不全」は初発症状出現から昏睡Ⅱ度以上の肝性脳症が出現するまでの期間が10日以内の「急性型」と，11日以降56日以内の「亜急性型」に分類する．

（注1）B型肝炎ウイルスの無症候性キャリアからの急性増悪例は「急性肝不全」に含める．また，自己免疫性で先行する慢性肝疾患の有無が不明の症例は，肝機能障害を発症する前の肝機能に明らかな低下が認められない場合は「急性肝不全」に含めて扱う．

（注2）アルコール性肝炎は原則的に慢性肝疾患を基盤として発症する病態であり，「急性肝不全」から除外する．但し，先行する慢性肝疾患が肥満ないしアルコールによる脂肪肝の症例は，肝機能障害の原因がアルコール摂取ではなく，その発症前の肝予備能に明らかな低下が認められない場合は「急性肝不全」として扱う．

（注3）薬物中毒，循環不全，妊娠脂肪肝，代謝異常など肝臓の炎症を伴わない肝不全も「急性肝不全」に含める．ウイルス性，自己免疫性，薬物アレルギーなど肝臓に炎症を伴う肝不全は「劇症肝炎」として扱う．

（注4）肝性脳症の昏睡度分類は犬山分類（1972年）に基づく（Table 2）．但し，小児では「第5回小児肝臓ワークショップ（1988年）による小児肝性昏睡の分類」を用いる（Table 3）．

（注5）成因分類は「難治性の肝疾患に関する研究班」の指針（2002年）を改変した新指針に基づく（Table 4）．

（注6）プロトロンビン時間が40%以下ないしはINR値1.5以上で，初発症状出現から8週以降24週以内に昏睡Ⅱ度以上の脳症を発現する症例は「遅発性肝不全」と診断し，「急性肝不全」の類縁疾患として扱う．

Table 2　肝性脳症の昏睡度分類（犬山シンポジウム：1972年）

昏睡度	精神症状	参考事項
Ⅰ	睡眠・覚醒リズムの逆転 多幸気分，ときに抑うつ状態 だらしなく，気にとめない状態	retrospectiveにしか判定できない場合も多い
Ⅱ	指南力（とき・場所）障害，物をとり違える（confusion） 異状行動（例：お金をまく，化粧品をゴミ箱にすてるなど） 時に傾眠傾向（普通の呼びかけで開眼し，会話ができる） 無礼な言動があったりするが，医師の指示には従う態度をみせる	興奮状態がない 尿，便失禁がない 羽ばたき振戦あり
Ⅲ	しばしば興奮状態，せん妄状態を伴い，反抗的態度をみせる 嗜眠傾向（ほとんど眠っている） 外的刺激で開眼しうるが，医師の指示には従わない，または従えない（簡単な命令には応じる）	羽ばたき振戦あり 指南力障害は高度
Ⅳ	昏睡（完全な意識の消失） 痛み刺激には反応する	刺激に対して，払いのける 動作，顔をしかめる
Ⅴ	深昏睡 痛み刺激に反応しない	

4. 考　　案

我が国の急性肝不全では，欧米と異なってアセトアミノフェン中毒が稀である．このことは劇症肝炎ないしLOHF以外の急性肝不全に関する全国調査でも確認された．なお，同調査では，劇症肝炎以外の急性肝不全ではアルコール性肝炎が最も多いが，その病態はacute-on-chronicであり，劇症肝炎とは病態が異なることが明らかになった．また，循環不全などの急性肝不全は昏睡を伴わない症例も存在するが，死亡例でも死因は合併症の症例が多く，肝不全の程度は軽度の場合が多いことが判明した．一方，プロトロンビン時間測定キットに関する調査では，劇症肝炎の診断基準で採用している40%に相当するINRは1.6から2.12とキットによって多彩であり，従来の劇症肝炎を網羅できる基準を設定するためには，INRを1.5以上と設定するのが妥当であると考えられた．

以上より，我が国における急性肝不全の診断基準としては，プロトロンビンのINRが1.5以上の症例を対

Table 3　小児肝性昏睡の分類（第5回小児肝臓ワークショップ：1988年）

意識障害（昏睡度）	年長児	乳児
I	いつもより元気がない	声を出して笑わない
II	傾眠傾向でおとなしい 見当識障害がある	あやしても笑わない 母親と視線が合わない（生後3カ月以降）
III	大きな声で呼ぶとかろうじて開眼する	
IV	痛み刺激でも覚醒しないが，顔をしかめたり，払いのけようとしたりする	
V	痛み刺激に全く反応しない	

Table 4　急性肝不全の成因分類

I. ウイルス性
　I-① A型
　I-② B型
　　I-②-1. 急性感染例
　　I-②-2. キャリア例*
　　I-②-2-i.　無症候性キャリア例（誘因なし）
　　I-②-2-ii.　無症候性キャリアの再活性化例
　　I-②-2-iii.　既往感染の再活性化例（de novo 肝炎）
　　I-②-3. 判定不能例
　I-③ C型
　I-④ E型
　I-⑤ その他
II. 自己免疫性
III. 薬物性
　III-① 薬物アレルギー
　III-② 薬物中毒
IV. 循環障害
V. 悪性腫瘍の肝浸潤
VI. 代謝性
VII. 術後肝不全
VIII. その他
IX. 成因不明
X. 分類不能

I，II，III-①およびIXは「劇症肝炎」に相当する急性肝不全の成因である．一方，III-②，IV～VIIIは肝臓に炎症を伴わない急性肝不全に相当する．なお，これら分類に際して用いる診断基準は別途定める．
*無症候性キャリアで免疫抑制・化学療法が誘因で発症した場合は再活性化例として扱う．また，HBs抗原陰性の既往感染例も再活性化した場合はキャリア例として扱うが，その位置づけに関しては，今後検討することにする．