CYP2A6 and the plasma level of 5-chloro-2, 4-dihydroxypyridine are determinants of the pharmacokinetic variability of tegafur and 5-fluorouracil, respectively, in Japanese patients with cancer given S-1

Ken-ichi Fujita,[1,2] Wataru Yamamoto,[1] Shoji Endo,[1] Hisashi Endo,[1] Fumio Nagashima,[1] Wataru Ichikawa,[1] Ryuhei Tanaka,[1] Toshimichi Miya,[1] Kazuhiro Araki,[1] Keiji Kodama,[1] Yu Sunakawa,[1] Masaru Narabayashi,[1] Keisuke Miwa,[1] Yuichi Ando,[1] Yuko Akiyama,[1,2] Kaori Kawara,[1] Tetsuya Kamataki[1] and Yasutsuna Sasaki[1,2,3]

[1]Department of Medical Oncology, Comprehensive Cancer Center, Saitama Medical University, 1397-1 Yamane, Hidaka, Saitama, 350-1298; [2]Project Research Laboratory, Research Center for Genomic Medicine, Saitama Medical University, 1397-1 Yamane, Hidaka, Saitama, 350-1241, Japan

(Received November 20, 2007/Revised December 22, 2007; January 9, 2008/Accepted January 16, 2008/Online publication March 28, 2008)

S-1 is an oral anticancer agent composed of tegafur (FT), 5-chloro-2,4-dihydroxypyridine (CDHP), and potassium oxonate. CDHP is added to prevent degradation of 5-fluorouracil (5-FU) by inhibiting dihydropyrimidine dehydrogenase. CYP2A6 is involved in the biotransformation of FT to 5-FU. Thus, we prospectively analyzed the effects of the *CYP2A6* genotype, plasma level of CDHP, and patient characteristics on the pharmacokinetic (PK) variability of FT and 5-FU. Fifty-four Japanese patients with metastatic or recurrent cancers who received S-1 were enrolled. The *CYP2A6* polymorphisms (4A*, **7*, and **9*) with deficient or reduced activity were analyzed. All subjects were classified into three groups according to their *CYP2A6* genotype: wild type (**1/*1*), one-variant allele (**1/any*), or two-variant alleles (combination other than **1*). The PK of FT, 5-FU, and CDHP were measured on day 1 of treatment. Multivariate regression analysis revealed that oral clearance of FT was associated with the *CYP2A6* genotype (analysis of variance [ANOVA], $P = 0.000838$). The oral clearance of FT seen in patients with the two-variant alleles was significantly lower than those in wild type and the one-variant allele (95% confidence intervals 0.75–2.41 and 0.41–1.82, respectively; Tukey-Kramer test). The area under the time–concentration curve (AUC) of 5-FU was significantly correlated with the AUC of CDHP (ANOVA, $P = 0.00126$). The AUC of 5-FU and CDHP were inversely correlated with creatinine clearance (ANOVA, $P = 0.0164$ and $P = 0.000762$, respectively). Although the *CYP2A6* variants are the cause of the PK variability of FT, the AUC of CDHP affected by renal function is the key determinant of the variability in the PK of 5-FU. (*Cancer Sci* 2008; 99: 1049–1054)**

S-1 (Taiho Pharmaceutical, Tokyo, Japan) is an oral anticancer agent. This anticancer drug is currently one of the most widely prescribed agents for treatment of gastric cancer in Japan, as a standard option for chemotherapy.[1–3] S-1 is a formulation of FT, CDHP, and Oxo in a molar ratio of 1:0.4:1.[4] FT is a pro-drug for cytotoxic 5-FU. The biotransformation of FT to 5-FU is demonstrated to be catalyzed by the liver drug-metabolizing enzyme CYP2A6.[5,6] The addition of CDHP increases the plasma level of 5-FU, as CDHP prevents degradation of 5-FU by competitively inhibiting DPD,[7] which is a rate-limiting enzyme responsible for 5-FU detoxification.[8] Oxo reduces the gastrointestinal toxicity caused by the active 5-FU by blocking the orotate phosphoribosyltransferase pathway, which relates to further activation of 5-FU.[9,10]

CYP2A6 is a polymorphic enzyme that shows considerable interindividual variability in its activity.[11–18] *CYP2A6*1* is defined as the wild-type allele. *CYP2A6*4* is a complete deletion of the *CYP2A6* gene.[11–13] Among the *CYP2A6*4* variants, *CYP2A6*4A* is a major variant seen in the Japanese population.[12,13] *CYP2A6*7* is a single nucleotide polymorphism (1412T>C) causing an amino acid change (I471T) that decreases enzymatic activity.[14] *CYP2A6*9 has* a –48T>G nucleotide substitution in the TATA box of the 5′ flanking region of the *CYP2A6* gene, which reduces the expression levels of CYP2A6 mRNA and protein in human livers.[15,16] These *CYP2A6* polymorphisms are seen frequently in Japanese people with allele frequencies of approximately 20% for *CYP2A6*4A*, 6% for *CYP2A6*7*, and 20% for *CYP2A6*9*.[13,17,18] Thus, it was postulated that these *CYP2A6* genetic polymorphisms in Japanese people influenced the PK variability of FT and 5-FU and susceptibility to adverse effects as well as S-1 anticancer activity. The results obtained by Daigo *et al*. partly support this hypothesis.[19] They found that the four-fold higher AUC of FT seen in one patient compared with four other patients was attributed to the simultaneous presence of *CYP2A6*4A* and *CYP2A6*11* (S224P), causing reduced catalytic activity in the patient. This implies that *CYP2A6* genetic polymorphisms alter the PK of FT.

Because CDHP has been reported to be predominantly excreted in the urine,[20] interindividual variability of the plasma level of CDHP caused by renal function was expected to occur. The variability in the plasma concentration of CDHP that thus occurred was assumed to affect the PK of 5-FU. Ikeda *et al*. have suggested this point with data obtained from four patients.[21]

Taking these considerations into account, the PK of 5-FU were expected to be influenced by polymorphisms in the *CYP2A6* gene,[11–18] and the PK of CDHP. Therefore, in the present study, the effects of *CYP2A6* genotype and plasma

[3]To whom correspondence should be addressed. E-mail: ysasaki@saitama-med.ac.jp

Abbreviations: ANOVA, analysis of variance; AUC, area under the time–concentration curve; $AUC_{0-\infty}$, area under the time–concentration curve from time zero to infinity; AUC_{0-8}, area under the time–concentration curve from time zero to 8 h; BSA, body surface area; Ccr, creatinine clearance; CDHP, 5-chloro-2,4-dihydroxypyridine; CL/F, oral clearance; DPD, dihydropyrimidine dehydrogenase; *DPYD*, dihydropyrimidine dehydrogenase; FT, tegafur; 5-FU, 5-fluorouracil; HPLC, high-performance liquid chromatography; HSD, honestly significant difference; Oxo, potassium oxonate; PCR, polymerase chain reaction; PK, pharmacokinetics.

Correction added 7 April 2008 after online publication: "$AUC_{0-\infty}$ of 5-FU" has been corrected to "AUC_{0-8} of 5-FU".

doi: 10.1111/j.1349-7006.2008.00773.x

CDHP levels on the PK variability of FT and 5-FU were examined prospectively.

Materials and Methods

Eligibility. All patients of 20 years or older with metastatic or recurrent and histologically confirmed solid tumors who received S-1, had a World Health Organization (WHO) performance status of 0–3, and no history of chemotherapy or radiotherapy within 4 weeks were eligible. Each patient was confirmed to have adequate bone marrow function (neutrocyte count, at least 1.5×10^9/L; platelet count, at least 100×10^9/L), liver function (serum bilirubin level, less than 3.0 mg/dL; transaminases, less than 2.0 times the upper limit of normal), and renal function (serum creatinine level, less than 2.0 mg/dL). All patients were asked for written informed consent 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. 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' BSA according to the manufacturer's package insert as distributed in Japan. The dose was 80 mg/day for patients with a BSA of less than 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 more than 1.5 m^2.

***CYP2A6* genotyping.** Genomic DNA was extracted from 200 μL peripheral blood, which had been stored at –80°C until analysis, with the use of a QIAamp Blood Kit (Qiagen, Hilden, Germany).

*CYP2A6*4A* was analyzed with the PCR restriction fragment length polymorphism method described by Nakajima *et al*.[12]

*CYP2A6*7* was analyzed with the allele-specific PCR-based method described by Ariyoshi *et al*.[14] with some modifications. Briefly, the first PCR was carried out with the following cycles. After initial denaturation at 94°C for 15 min, amplification was carried out by denaturation at 94°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 2 min for 32 cycles. The product of the first PCR was diluted 10-fold with distilled water. The diluted product was subjected to a second PCR. The reaction mixture contained 0.25 mM dNTP in a final volume of 25 μL. After initial denaturation at 94°C for 15 min, amplification was carried out by denaturation at 94°C for 20 s, annealing at 60°C for 20 s, and extension at 72°C for 45 s for 13 cycles.

*CYP2A6*9* was analyzed with the allele-specific PCR-based method reported by Yoshida *et al*.[15] with minor changes. After initial denaturation at 94°C for 15 min, PCR amplification was carried out by denaturation at 94°C for 30 s, annealing at 64°C for 30 s, and extension at 72°C for 25 s for 25 cycles.

Determination of FT and 5-FU. Blood samples for PK analysis were obtained on the first day of treatment. Blood samples were taken right before administration of S-1 and 0.5, 1, 2, 4, 8, and 24 h after the first administration. The second dose of S-1 on that day was skipped for the 24 h of PK analysis of the first dose. The samples were centrifuged immediately, and plasma was stored at –80°C until analysis.

Plasma concentrations of FT and 5-FU were analyzed by a HPLC method reported previously,[22,23] with modifications. A 250-μL plasma sample was mixed with 100 μL of 0.2 M phosphate buffer (pH 7.0) and 100 μL of 100 μM 5-bromouracil (internal standard). After adding 4 mL ethyl acetate, the mixture was shaken for 10 min and centrifuged at 2300*g* for 10 min. The organic layer was transferred to another test tube. This extraction step was repeated once. The organic layers were combined and evaporated to dryness. The residue was dissolved in 0.3 mL of 20 mM $NaClO_4$ (pH 2.5) and centrifuged at 20 630*g* for 10 min. Then, a 100-μL portion of the supernatant was applied to a HPLC system (Hitachi model 7000 series; Hitachi, Tokyo, Japan), equipped with a Capcell Pak C18 SG120 analytical column (4.6 × 250 mm; 5 μm; Shiseido, Tokyo, Japan). HPLC was carried out at 35°C at a flow rate of 0.8 mL/min. The mobile phase consisted of 20 mM $NaClO_4$ (pH 2.5) (solvent A) and acetonitrile (solvent B). A 20-min run was carried out with a linear gradient of 100 to 87.5% for solvent A. The eluent was monitored at 270 nm. Quantification of FT and 5-FU was achieved by comparing the respective peak areas on a chromatogram with that of an internal standard, 5-bromouracil.

The quantification limits were 50 ng/mL (0.25 μM) for FT and 16 ng/mL (0.125 μM) for 5-FU. The intra-assay and interassay coefficients of variation were 5.15 and 8.93% for FT and 6.41 and 6.31% for 5-FU, respectively.

Determination of CDHP. The plasma concentrations of CDHP were determined with a gas chromatography-negative ion chemical ionization mass spectrometry system (Falco Biosystems, Kyoto Japan) as described by Matsushima *et al*.[22] The quantification limit of CDHP was 2 ng/mL (13.8 nM). The intra-assay and interassay coefficients of variation were confirmed to be less than 10%.

Pharmacokinetic parameters. The AUC (μM·h) of FT, 5-FU, and CDHP were calculated with the linear trapezoidal rule (until the peak plasma concentration) and linear-log trapezoidal rule (until the last measurable concentration), using a computer program (WinNonlin version 5.1 software; Pharsight Corporation, Mountain View, CA, USA). The CL/F (L/h) of FT was also determined.

Statistical analysis. Allele and genotype frequencies for each polymorphic allele in the *CYP2A6* gene were determined using SNPAlyze 5.1 (Dynacom, Yokohama, Japan). The significance of deviations from Hardy–Weinberg equilibrium was tested with the program SNPAlyze 5.1. Linkage disequilibrium analysis to calculate the correlation coefficient r^2 and disequilibrium parameter (D′) between *CYP2A6*7* and the *CYP2A6*9* was also carried out using SNPAlyze 5.1.

Correlations of the PK of FT or 5-FU with possibly related factors were examined by ANOVA. Correlations were considered statistically significant when the two-tailed *P*-value was less than 0.05. The Tukey–Kramer HSD test was used to examine the possible correlation between the CL/F of FT and the *CYP2A6* genotypes. The correlations were also analyzed by multivariate linear least-squares regression analysis. The estimated model was considered to be significant when the two-tailed *P*-value obtained by ANOVA was less than 0.05. Factors in the model were significantly associated with a variable when the two-tailed *P*-value was less than 0.05. All analyses were carried out with JMP version 6 software (SAS Institute, Cary, NC, USA).

Results

Patient characteristics. A total of 54 Japanese patients at Saitama Medical University were enrolled in the present study from September 2005 through April 2007. The patient characteristics are summarized in Table 1. Most of the patients showed performance status 0 or 1. The median of the Ccr calculated with the Cockcroft–Gault equation was 81.5 mL/min, ranging from 39 to 174 mL/min. The most frequent tumor was gastric cancer.

***CYP2A6* genotypes.** The allele frequencies of *CYP2A6*4A*, **7*, and **9* were 20.4, 20.4, and 17.6%, respectively. All of the polymorphic alleles were in Hardy–Weinberg equilibrium ($P > 0.05$). The allele frequencies of *CYP2A6*4A* and **9* were similar to those reported previously,[13,17,18] whereas that of **7* was somewhat higher. The distribution of *CYP2A6* genotypes was as follows: **1/*1*, 22.2%; **1/*4A*, 14.8%; **1/*7*, 7.4%; **1/*9*, 16.7%; **4A/*4 A*, 5.6%; **4A/*7*, 11.1%; **4A/*9*, 3.7%; **7/*7*, 7.4%; **7/*9*, 7.4%, and **9/*9*, 3.7%. As linkage between *CYP2A6*7* and *CYP2A6*9* was not observed ($D' = -1$, $r^2 = 0.0546$), haplotype analysis was not carried out.

doi: 10.1111/j.1349-7006.2008.00773.x

Table 1. Patient characteristics

Characteristic	Number of patients
Age (years)†	
61 (33–85)	54
Sex	
Male	34
Female	20
Performance status	
0	29
1	23
2≤	2
Creatinine (mg/dL)†	
0.67 (0.43–1.2)	54
Creatinin clearance (mL/min)†‡	
81.5 (39–174)	54
Total bilirubin (mg/dL)†	
0.5 (0.2–1.5)	54
Tumor type	
Stomach	32
Colon	9
Breast	6
Other	7
Prior chemotherapy regimens	
0	32
1	12
2	10

†Values are expressed as medians, with ranges in parentheses.
‡Creatinine clearance was calculated with the Cockcroft–Gault equation.

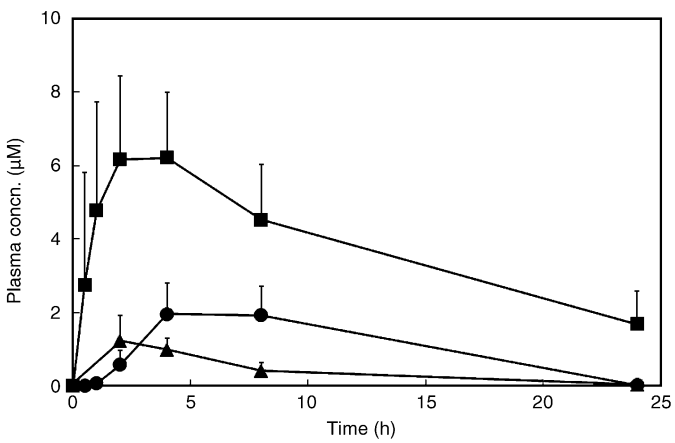

Fig. 1. Pharmacokinetic profiles of tegafur (FT), 5-fluorouracil (5-FU), and 5-chloro-2,4-dihydroxypyridine (CDHP) in 54 patients. (■), FT; (●), 5-FU; (▲), CDHP. Data points and bars represent the mean and SD, respectively.

Pharmacokinetic profiles of FT, 5-FU, and CDHP. The PK profiles of FT, 5-FU, and CDHP are shown in Figure 1. The $AUC_{0-\infty}$ of FT was calculated with time points from 0 to 24 h. The mean ± SD for the $AUC_{0-\infty}$ of FT was 116 ± 51.2 μM·h. The median was 106 μM·h (range, 35.1–297 μM·h). CL/F was calculated from the $AUC_{0-\infty}$ and the dose of FT. The mean ± SD for the CL/F was 2.76 ± 1.14 L/h. The median was 2.68 L/h (range 0.99–7.13 L/h). In 51 out of 54 cases, the plasma concentrations of 5-FU at 24 h were under the detection limit. Because the elimination phase of 5-FU was not observed until 8 h, the $AUC_{0-\infty}$ of 5-FU could not be calculated in many cases. Therefore, the AUC_{0-8} was calculated for 5-FU. The mean ± SD for the AUC_{0-8} of 5-FU was 10.5 ± 3.70 μM·h. The median was 9.84 μM·h (range 4.16–20.8 μM·h). The $AUC_{0-\infty}$ of CDHP was determined with time points of 0, 2, 4, 8, and 24 h. The mean ± SD for the $AUC_{0-\infty}$ of CDHP was 8.35 ± 3.12 μM·h. The median was 9.84 μM·h (range 4.16–20.8 μM·h). Thus, the PK profiles of FT, 5-FU, and CDHP still varied among patients, even if the S-1 doses were fixed by BSA.

Table 2. Relationship between the pharmacokinetics of tegafur (FT) or 5-fluorouracil (5-FU) and possible factors

	R^2	P
CL/F of FT		
$AUC_{0-\infty}$ of CDHP	0.0039	0.655
BSA	0.0024	0.727
Age	0.024	0.265
Total bilirubin	0.0051	0.609
CYP2A6 genotype	0.33	0.0000333
Sex	0.038	0.159
AUC_{0-8} of 5-FU		
$AUC_{0-\infty}$ of CDHP	0.30	0.0000211
BSA	0.0036	0.666
Age	0.0039	0.656
Total bilirubin	0.041	0.140
CYP2A6 genotype	0.026	0.515
Sex	0.0020	0.748

AUC, area under the concentration–time curve; BSA, body surface area; CDHP, 5-chloro-2,4-dihydroxypyridine; CL/F, oral clearance.
The respective relationships were analyzed by ANOVA.

Factors affecting the CL/F of FT. The effects of factors including the *CYP2A6* genotype, plasma level of CDHP, and patient characteristics on the CL/F of FT were examined. As mentioned, the variant *CYP2A6* alleles were assumed to generate transcripts possessing lower or no enzymatic activity. Thus, all subjects in the present study were classified into three groups according to *CYP2A6* genotype: wild type (**1/*1*), one-variant allele (**1/*4A*, **1/*7* or **1/*9*), or two-variant alleles (**4A/*4A*, **4A/*7*, **4A/*9*, **7/*7*, **7/*9* or **9/*9*). The CL/F of FT was found to be significantly associated with the *CYP2A6* genotype (ANOVA, $P = 0.0000333$, $R^2 = 0.33$) (Table 2). The CL/F of FT seen in patients with the two-variant alleles was significantly lower than those with wild type or one-variant allele (95% confidence intervals 0.75–2.41 and 0.41–1.82, respectively; Tukey-Kramer HSD test) (Fig. 2a). A significant association between the CL/F of FT and the respective 10 *CYP2A6* genotypes was also observed (ANOVA, $P = 0.000363$, $R^2 = 0.48$). Comparisons for all genotype pairs using the Tukey–Kramer HSD test revealed that the CL/F of FT seen in patients with **4/*4*, **4/*7* or **7/*7*, but not with **4/*9*, **7/*9* or **9/*9*, was significantly lower than that seen in patients with **1/*1*. Multivariate regression analysis indicated that the *CYP2A6* genotype could be a significant predictor of the CL/F of FT (ANOVA, $P = 0.000838$, $R^2 = 0.40$) (Table 3). The *P*-values for the estimated parameters of the subjects carrying wild type and two-variant alleles of the *CYP2A6* genotypes were 0.00329 and 0.0000261, respectively.

Factors influencing the AUC of 5-FU. Next, factors that might influence the variability of the AUC of 5-FU were examined. The AUC_{0-8} of 5-FU was correlated significantly with the $AUC_{0-\infty}$ of CDHP (ANOVA, $P = 0.0000211$, $R^2 = 0.30$) (Table 2; Fig. 3). Even when the relationship was analyzed by excluding the data from a patient who showed the highest AUC of 5-FU and CDHP, a statistically significant correlation between the AUC of 5-FU and CDHP was still observed (ANOVA, $P = 0.0191$, $R^2 = 0.17$). The AUC_{0-8} of 5-FU did not correlate with other factors including *CYP2A6* genotype (Table 2; Fig. 2b). No association between the AUC_{0-8} of 5-FU and the respective 10

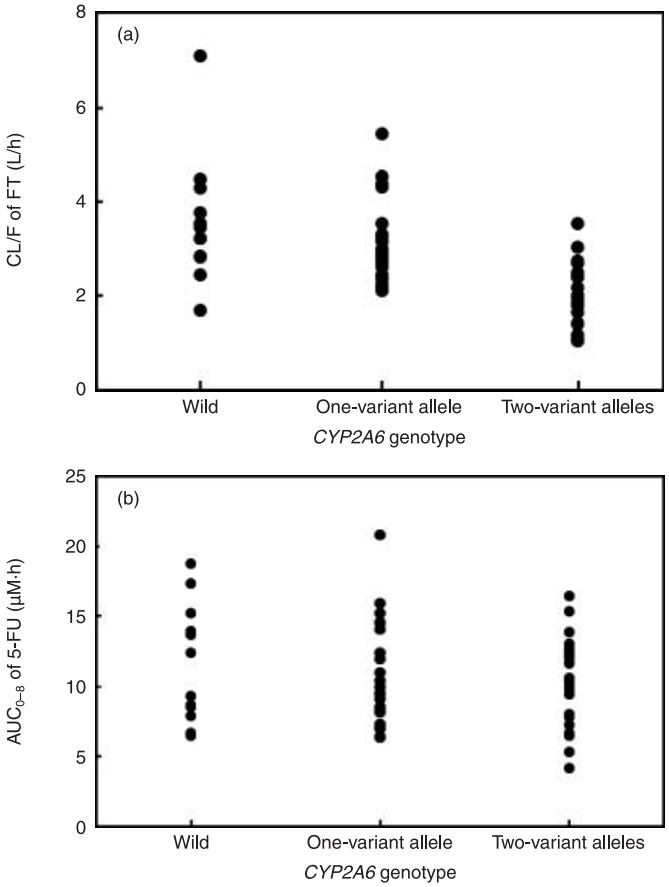

Fig. 2. Pharmacokinetics of tegafur (FT) or 5-fluorouracil (5-FU) and the *CYP2A6* genotype. (a) Oral clearance (CL/F) of FT and the *CYP2A6* genotype. The mean ± SD of the CL/F observed in patients with wild type, one-variant allele, and two-variant alleles was 3.56 ± 1.36, 3.10 ± 0.89, and 1.98 ± 0.69 L/h, respectively. (b) Area under the time–concentration curve (AUC) of 5-FU and the *CYP2A6* genotype. The mean ± SD of the AUC of 5-FU seen in patients with wild type, one-variant allele, and two-variant alleles was 11.5 ± 4.21, 10.5 ± 3.81, and 9.98 ± 3.32 μM·h, respectively.

CYP2A6 genotypes was also observed (ANOVA). Multivariate regression analysis suggested that the $AUC_{0-\infty}$ of CDHP was a good predictive factor of the AUC_{0-8} of 5-FU (ANOVA, $P = 0.00126$, $R^2 = 0.39$) (Table 3). The *P*-value for the estimated parameter of $AUC_{0-\infty}$ of CDHP was 0.0000129. When the multivariate regression analysis was carried out without the data from a patient who showed the highest AUC of 5-FU and CDHP, the *P*-value obtained by ANOVA was 0.073. It was not clear whether total bilirubin was a significant factor related to the AUC_{0-8} of 5-FU (Tables 2,3).

Effects of Ccr on the AUC of 5-FU or CDHP. As shown in Figure 4, the AUC_{0-8} of 5-FU correlated inversely with Ccr (ANOVA, $P = 0.0164$, $R^2 = 0.11$). The inverse correlation between the $AUC_{0-\infty}$ of CDHP and Ccr was also observed (ANOVA, $P = 0.000762$, $R^2 = 0.20$). One patient with a low Ccr (45 mL/min) showed the highest AUC_{0-8} of 5-FU and $AUC_{0-\infty}$ of CDHP. It should be noted that the patient suffered from grade 3 stomatitis, fatigue, and diarrhea and died after 5 days of S-1 treatment (Figs 3,4).

Discussion

In the present study, we found that the plasma level of CDHP, but not the *CYP2A6* genotype, is the key rate-limiting step in the S-1 disposition. It seems likely that CDHP makes the degradation of 5-FU by DPD the rate-limiting step through competitive inhibition, compared to the formation of 5-FU from FT by CYP2A6.

Of interest, the tolerable dose of S-1 has been known to be substantially higher in Japanese patients than in Western patients.[24–27] So far, ethnically different distributions in *CYP2A6* genetic polymorphisms have been thought to be

Table 3. Relationship between the pharmacokinetics of tegafur (FT) or 5-fluorouracil (5-FU) and possible factors estimated by multivariate regression analysis

	Term	Estimated parameter	*P*
CL/F of FT	Intercept	4.2	0.0908
($R^2 = 0.40$, $P = 0.000838$)	*CYP2A6* genotype†		
	Wild	0.67	0.00329
	One-variant	0.22	0.233
	Two-variants	–0.89	0.0000261
	$AUC_{0-\infty}$ of CDHP	–0.038	0.397
	BSA	–0.26	0.828
	Age	–0.012	0.418
	Sex†		
	Male	0.27	0.120
	Total bilirubin	0.18	0.745
AUC_{0-8} of 5-FU	Intercept	5.2	0.513
($R^2 = 0.39$, $P = 0.00126$)	*CYP2A6* genotype†		
	Wild	0.84	0.244
	One-variant	–0.72	0.235
	Two-variants	–0.12	0.853
	$AUC_{0-\infty}$ of CDHP	0.71	0.0000129
	BSA	–0.18	0.963
	Age	–0.036	0.471
	Sex†		
	Male	–0.028	0.960
	Total bilirubin	3.8	0.0431

AUC, area under the concentration–time curve; BSA, body surface area; CDHP, 5-chloro-2,4-dihydroxipyridine; CL/F, oral clearance.
†The summation of estimated parameters for each category of nominal term was calculated to be one (JMP version 6).

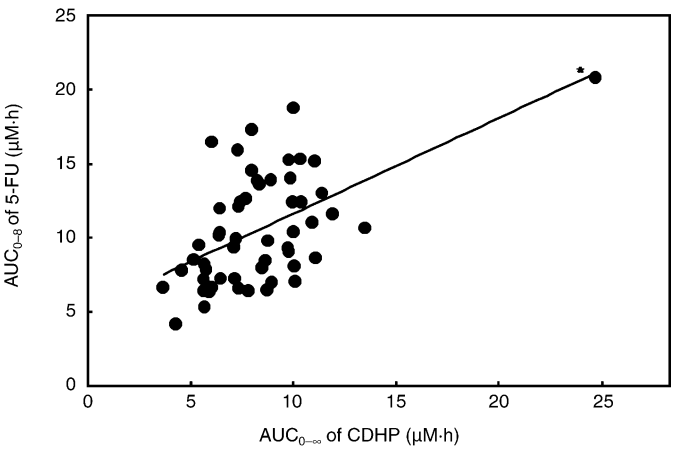

Fig. 3. Area under the time–concentration curve (AUC) of 5-fluorouracil (5-FU) and 5-chloro-2,4-dihydroxypyridine (CDHP) exposure. *The patient showed grade 3 stomatitis, fatigue, and diarrhea and died after 5 days of S-1 treatment. The mean ± SD of the AUC of 5-FU and CDHP was 10.5 ± 3.70 and 8.35 ± 3.12 μM·h, respectively.

doi: 10.1111/j.1349-7006.2008.00773.x

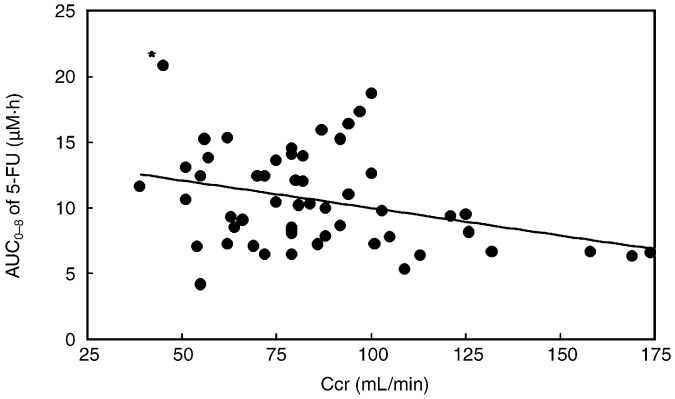

Fig. 4. Area under the time–concentration curve (AUC) of 5-fluorouracil (5-FU) and creatinine clearance (Ccr). *The patient suffered from grade 3 stomatitis, fatigue, and diarrhea and died after 5 days of S-1 treatment.

involved in the difference in the average PK of 5-FU and discordant outcomes between Japanese and white people.[24,26] Because the frequency of the mutant *CYP2A6* alleles is higher in Japanese people than in white people,[13,17,18] it is reasonable to postulate that the average catalytic activity of CYP2A6 to metabolize FT is lower in Japanese people. Therefore, when a comparable dose of S-1 is administered to both ethnic groups, the average plasma level of 5-FU in Japanese people may be lower than in white people. However, because the *CYP2A6* genotype was not associated with the AUC of 5-FU (Table 2; Fig. 2b), the lower average formation of 5-FU from FT might not be the cause for the lower average AUC of 5-FU in Japanese people. From the results that the AUC of 5-FU significantly correlated with the AUC of CDHP (Fig. 3), another hypothesis is possible. If a comparable dose of S-1 (mg/m^2) is administered to Japanese and white people, the average AUC of CDHP seen in Japanese people might be lower than that seen in white people, as the average BSA of Japanese is lower.[28,29] The average actual dose of CDHP (mg/body) is probably lower in Japanese people than in white people. The average lower plasma level of CDHP in Japanese people might result in the lower inhibition of DPD, causing higher degradation of 5-FU and leading to a higher average dose of S-1. Further studies with Western patients are needed.

Because CDHP has been reported to be predominantly excreted in the urine,[20] renal function might be a key factor determining the PK of CDHP as well as 5-FU.[21] Our results showed that impaired renal function caused the high plasma concentrations of CDHP and 5-FU. As shown in Figures 3 and 4, a patient with low Ccr (45 mL/min) showed the highest $AUC_{0–8}$ of 5-FU and $AUC_{0–\infty}$ of CDHP. Of particular importance, the patient suffered from grade 3 stomatitis, fatigue, and diarrhea and died after 5 days of S-1 treatment. It is necessary to clarify the relationship between renal function (Ccr) and S-1-induced toxicity.

In the present study, each S-1 dose was adjusted based on BSA (40–60 mg/body twice daily). However, the $AUC_{0–8}$ of 5-FU still varied (Fig. 1). This implies that BSA-based dosing is not sufficient to reduce the interpatient variability in the AUC_{0-8} of 5-FU. Because the $AUC_{0–8}$ of 5-FU was inversely correlated with Ccr (Fig. 4), the conventional BSA-based dosing would be compensated by Ccr to reduce the interindividual PK variability of 5-FU.

We extrapolated the AUC of FT and CDHP to $AUC_{0–\infty}$ from the plasma concentration data obtained from time 0–24 h. This extrapolation seemed to be reasonable as we obtained similar relationships shown in Tables 2 and 3 by using the $AUC_{0–24}$ of FT and CDHP (data not shown).

According to the results obtained by Hirata *et al*. the elimination phase of 5-FU was observed until 8 h in 11 of 12 patients.[20] Considering their results, we decided to calculate the $AUC_{0–\infty}$ of 5-FU from the plasma concentration data obtained at 0, 0.5, 1, 2, 4, 8, and 24 h. However, in the present study, we could not necessarily observe the elimination phase of 5-FU until 8 h. Most patients enrolled in this study showed a PK profile of 5-FU similar to one patient reported in Hirata's study whose elimination phase of 5-FU was observed after 8 h.[20] Therefore, we calculated the $AUC_{0–8}$ of 5-FU, instead of the $AUC_{0–\infty}$.

Diasio *et al*.[30] demonstrated that a heritable defect in DPD, which is a rate-limiting drug-metabolizing enzyme involved in the detoxification of 5-FU,[31] can cause 5-FU-related severe toxicity. Some polymorphisms in *DPYD* have been shown to be associated with reduced DPD activity and 5-FU toxicity. In the present study, *DPYD*11* and *DPYD*12*, previously found in Japanese and related to severe 5-FU-related toxicity,[32] were not found. As expected, *DPYD*2* and *DPYD*3*, which were also reported to be related with severe toxicity of 5-FU in white people,[33] were not found.

The present study might be regarded as exploratory as the sample size was relatively small and the results have not been corrected for multiple comparisons. Further studies with large sample sizes need to be carried out to confirm the results shown in this study. In conclusion, we propose that *CYP2A6* genotype analysis might be useful to evaluate the variability in the plasma levels of FT, whereas measurement of the total exposure of CDHP, which is in part affected by Ccr, is critical to determine the PK of 5-FU.

Acknowledgments

We thank Dr Masahiko Ando at Kyoto University Health Service for valuable advice on the statistical analyses. This study was supported in part by a Grant-in-Aid for Cancer Research (17-8) and Health and Labour Sciences Research Grant (2005-Clinical Cancer Research-008) from the Ministry of Health, Labour, and Welfare of Japan, by a Grant-in-Aid for Scientific Research (A) (16200038) from Japan Society for the Promotion of Science and by a grant from Japan Research Foundation for Clinical Pharmacology.

References

1 Ohtsu A, Yoshida S, Saijo N. Disparities in gastric cancer chemotherapy between the East and West. *J Clin Oncol* 2006; **24**: 2188–96.
2 Boku N, Yamamoto K, Shirao T *et al*. Randomized phase III study of 5-fluorouracil (5-FU) alone versus combination of irinotecan and cisplatin (CP) versus S-1 alone in advanced gastric cancer (JCOG9912). *J Clin Oncol* 2007; **25**: 965S.
3 Narahara H, Koizumi W, Hara T *et al*. Randomized phase III study of S-1 alone versus S-1 + cisplatin in the treatment for advanced gastric cancer (The SPIRITS trial) *SPIRITS*. S-1 plus cisplatin vs S-1 in RCT in the treatment for stomach cancer. *J Clin Oncol* 2007; **25**: 201S.
4 Shirasaka T, Nakano K, Takechi T *et al*. Antitumor activity of 1 M tegafur–0.4 M 5-chloro-2,4-dihydroxypyridine–1 M potassium oxonate (S-1) against human colon carcinoma orthotopically implanted into nude rats. *Cancer Res* 1991; **56**: 2602–6.
5 Ikeda K, Yoshisue K, Matsushima E *et al*. Bioactivation of tegafur to 5-fluorouracil is catalyzed by cytochrome P450 2A6 in human liver microsomes in vitro. *Clin Cancer Res* 2000; **6**: 4409–15.
6 Komatsu T, Yamazaki H, Shimada N, Nakajima M, Yokoi T. Roles of cytochromes P450 1A2, 2A6, and 2C8 in 5-fluorouracil formation from tegafur, an anticancer prodrug, in human liver microsomes. *Drug Metab Dispos* 2000; **28**: 1457–63.
7 Tatsumi K, Fukushima M, Shirasaka T, Fujii S. Inhibitory effects of

pyrimidine, barbituric acid and pyridine derivatives on 5-fluorouracil degradation in rat liver extracts. *Jpn J Cancer Res* 1987; **78**: 748–55.
8 Shiotani T, Weber G. Purification and properties of dihydrothymine dehydrogenase from rat liver. *J Biol Chem* 1981; **256**: 219–24.
9 Shirasaka T, Shimamoto Y, Fukushima M. Inhibition by oxonic acid of gastrointestinal toxicity of 5-fluorouracil without loss of its antitumor activity in rats. *Cancer Res* 1993; **53**: 4004–9.
10 Takechi T, Nakano K, Uchida J *et al*. Antitumor activity and low intestinal toxicity of S-1, a new formulation of oral tegafur, in experimental tumor models in rats. *Cancer Chemother Pharmacol* 1997; **39**: 205–11.
11 Nunoya K, Yokoi T, Kimura K *et al*. A new deleted allele in the human cytochrome P450 2A6 (CYP2A6) gene found in individuals showing poor metabolic capacity to coumarin and (+)-cis-3,5-dimethyl-2-(3-pyridyl) thiazolidin-4-one hydrochloride (SM-12502). *Pharmacogenetics* 1998; **8**: 239–49.
12 Nakajima M, Yoshida R, Fukami T, McLeod HL, Yokoi T. Novel human CYP2A6 alleles confound gene deletion analysis. *FEBS Lett* 2004; **569**: 75–81.
13 Nakajima M, Fukami T, Yamanaka H *et al*. Comprehensive evaluation of variability in nicotine metabolism and CYP2A6 polymorphic alleles in four ethnic populations. *Clin Pharmacol Ther* 2006; **80**: 282–97.
14 Ariyoshi N, Sawamura Y, Kamataki T. A novel single nucleotide polymorphism altering stability and activity of CYP2a6. *Biochem Biophys Res Commun* 2001; **281**: 810–14.
15 Yoshida R, Nakajima M, Nishimura K, Tokudome S, Kwon JT, Yokoi T. Effects of polymorphism in promoter region of human CYP2A6 gene (CYP2A6*9) on expression level of messenger ribonucleic acid and enzymatic activity *in vivo* and *in vitro*. *Clin Pharmacol Ther* 2003; **74**: 69–76.
16 Kiyotani K, Yamazaki H, Fujieda M *et al*. Decreased coumarin 7-hydroxylase activities and CYP2A6 expression levels in humans caused by genetic polymorphism in CYP2A6 promoter region (CYP2A6*9). *Pharmacogenetics* 2003; **13**: 689–95.
17 Schoedel KA, Hoffmann EB, Rao Y, Sellers EM, Tyndale RF. Ethnic variation in CYP2A6 and association of genetically slow nicotine metabolism and smoking in adult Caucasians. *Pharmacogenetics* 2004; **14**: 615–26.
18 Fujita K. Cytochrome P450 and anticancer drugs. *Curr Drug Metab* 2006; **7**: 23–37.
19 Daigo S, Takahashi Y, Fujieda M *et al*. A novel mutant allele of the CYP2A6 gene (CYP2A6*11) found in a cancer patient who showed poor metabolic phenotype towards tegafur. *Pharmacogenetics* 2002; **12**: 299–306.
20 Hirata K, Horikoshi N, Aiba K *et al*. Pharmacokinetic study of S-1, a novel oral fluorouracil antitumor drug. *Clin Cancer Res* 1999; **5**: 2000–5.
21 Ikeda M, Furukawa H, Imamura H *et al*. Pharmacokinetic study of S-1, a novel oral fluorouracil antitumor agent in animal model and inpatients with impaired renal function. *Cancer Chemother Pharmacol* 2002; **50**: 25–32.
22 Matsushima E, Yoshida K, Kitamura R, Yoshida K. Determination of S-1 (combined drug of tegafur, 5-chloro-2,4-dihydroxypyridine and potassium oxonate) and 5-fluorouracil in human plasma and urine using high-performance liquid chromatography and gas chromatography-negative ion chemical ionization mass spectrometry. *J Chromatogr B* 1997; **691**: 95–104.
23 Komatsu T, Yamazaki H, Shimada N *et al*. Involvement of microsomal cytochrome P450 and cytosolic thymidine phosphorylase in 5-fluorouracil formation from tegafur in human liver. *Clin Cancer Res* 2001; **7**: 675–8.
24 Ajani JA, Faust J, Ikeda K *et al*. Phase I pharmacokinetic study of S-1 plus cisplatin in patients with advanced gastric carcinoma. *J Clin Oncol* 2005; **23**: 6957–65.
25 van Groeningen CJ, Peters GJ, Schornagel JH *et al*. Phase I clinical and pharmacokinetic study of oral S-1 in patients with advanced solid tumors. *J Clin Oncol* 2000; **18**: 2772–9.
26 Hoff PM, Saad ED, Ajani JA *et al*. Phase I study with pharmacokinetics of S-1 on an oral daily schedule for 28 days in patients with solid tumors. *Clin Cancer Res* 2003; **9**: 134–42.
27 Lenz HJ, Lee FC, Haller DG *et al*. Extended safety and efficacy data on S-1 plus cisplatin in patients with untreated, advanced gastric carcinoma in a multicenter phase II study. *Cancer* 2007; **109**: 33–40.
28 Nakajima Y, Yoshitani T, Fukushima-Uesaka H *et al*. Impact of the haplotype CYP3A4*16B harboring the Thr185Ser substitution on paclitaxel metabolism in Japanese patients with cancer. *Clin Pharmacol Ther* 2006; **80**: 179–91.
29 Baker SD, Verweij J, Rowinsky EK *et al*. Role of body surface area in dosing of investigational anticancer agents in adults, 1991–2001. *J Natl Cancer Inst* 2002; **94**: 1883–8.
30 Diasio RB, Beavers TL, Carpenter JT. Familial deficiency of dihydropyrimidine dehydrogenase. Biochemical basis for familial pyrimidinemia and severe 5-fluorouracil-induced toxicity. *J Clin Invest* 1988; **81**: 47–51.
31 Mukherjee KL, Boohar J, Wentland D, Ansfield FJ, Heidelberger C. Studies on fluorinated pyrimidines: XVI.Metabolism of 5-fluorouracil-2-C^{14} and 5-fluoro-2′-deoxyuridine-2-C^{14} in cancer patients. *Cancer Res* 1963; **23**: 49–66.
32 Kouwaki M, Hamajima N, Sumi S *et al*. Identification of novel mutations in the dihydropyrimidine dehydrogenase gene in a Japanese patient with 5-fluorouracil toxicity. *Clin Cancer Res* 1998; **4**: 2999–3004.
33 Ridge SA, Sludden J, Brown O *et al*. Dihydropyrimidine dehydrogenase pharmacogenetics in Caucasian subjects. *Br J Clin Pharmacol* 1998; **46**: 151–6.

doi: 10.1111/j.1349-7006.2008.00773.x