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ORIGINAL ARTICLE

Impact of *CYP3A4* haplotypes on irinotecan pharmacokinetics in Japanese cancer patients

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Abstract
Background and purpose Cytochrome P450 3A4 (CYP3A4) converts an anticancer prodrug, irinotecan, to inactive metabolites such as APC. However, the contribution of *CYP3A4* genetic polymorphisms to irinotecan pharmacokinetics (PK) and pharmacodynamics (PD) is not fully elucidated. In paclitaxel-administered cancer patients, an association of *CYP3A4*16B* harboring the low activity allele **16* [554C > G (Thr185Ser)] has been shown with altered metabolite/paclitaxel area under the plasma concentration–time curve (AUC) ratios, suggesting a possible impact of **16B* on the PK of other drugs. In this study, the effects of *CYP3A4* haplotypes including **16B* on irinotecan PK/PD were investigated in irinotecan-administered patients.
Methods The *CYP3A4* genotypes for 177 Japanese cancer patients who received irinotecan were defined in terms of

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4 major haplotypes, i.e., **1A* (wild type), **1G* (IVS10 + 12G > A), **16B* [554C > G (Thr185Ser) and IVS10 + 12G > A], and **18B* [878T > C (Leu293Pro) and IVS10 + 12G > A]. Associations of *CYP3A4* genotypes with irinotecan PK and severe toxicities (grade 3 diarrhea and grade 3 or 4 neutropenia) were investigated.

Results Area under the concentration–time curve ratios of APC/irinotecan, an in vivo parameter for CYP3A4 activity, were significantly higher in females than in males. The male patients with **16B* showed significantly decreased AUC ratios (APC/irinotecan) with 50% of the median value of the *non-*16B* male patients (no **16B*-bearing female patients in this study), whereas no significant alteration in the AUC ratios was observed in the patients with **18B*. A slight trend toward increasing AUC ratios (20%) was detected in both male and female patients bearing **1G*. Multivariate analysis confirmed contributions of *CYP3A4*16B* (coefficient ± SE = −0.18 ± 0.077, $P = 0.021$) and **1G* (0.047 ± 0.021, $P = 0.029$) to the AUC ratio. However, no significant association was observed between the *CYP3A4* genotypes and total clearance of irinotecan or toxicities (severe diarrhea and neutropenia).

Conclusion This study suggested that *CYP3A4*16B* was associated with decreased metabolism of irinotecan to APC. However, the clinical impact of *CYP3A4* genotypes on total clearance and irinotecan toxicities was not significant.

Keywords CYP3A4 · Haplotype · Irinotecan · Pharmacogenetics

Introduction

Human cytochrome P450 3A4 (CYP3A4) is a major CYP enzyme, abundant in the liver and intestine, and is involved in the metabolism of endogenous substances, including steroid hormones, and a variety of exogenous compounds such as environmental chemicals and pharmaceuticals. Large inter-individual differences in liver and intestinal CYP3A4 expression levels are known and thought to be caused by multiple factors including genetic variations, disease status, and modulation by exogenous stimuli, such as smoking, diet, and drugs [5, 18, 31]. The tissue-specific CYP3A4 expression is regulated by constitutive and inducible mechanisms via activation of the nuclear receptors, pregnane X receptor (PXR), constitutive androstane receptor (CAR), and vitamin D receptor (VDR) [5, 18]. Since approximately half of clinical drugs currently in use are metabolized by CYP3A4 [5, 33], it is important to find suitable biomarkers, including genetic polymorphisms, which can reflect in vivo CYP3A4 activity and predict individual responses to CYP3A4-metabolized drugs. Recent progress in pharmacogenetic research has led to the accumulation of knowledge about *CYP3A4* genetic variations responsible for altered expression or function. To date, more than 30 *CYP3A4* variations have been identified (http://www.cypalleles.ki.se/cyp3a4.htm), and large ethnic differences in their frequencies have been recognized. *CYP3A4*1B* (−392A > G), a single nucleotide polymorphism (SNP) in the 5′-flanking region, is found in Caucasians (2–9.6%) and African–Americans (35–67%), but not in Asians [16]. As relatively frequent coding SNPs, **2* [664T > C (Ser222Pro)] (2.7%) and **17* [566T > C (Phe189Ser)] (2%) were detected in Caucasians; **10* [520G > C (Asp174His)] in Caucasians (0.24–2%) and Mexicans (5%); **15* [485G > A (Arg162Gln)] (2–4%) in African–Americans; **16* [554C > G (Thr185Ser)] in East Asians (1.4–5%) and Mexicans (5%); **18* [878T > C (Leu293Pro)] (2.3–10%) in East Asians [2, 4, 17, 24]. We previously identified 25 *CYP3A4* haplotypes in a Japanese population [4]. The haplotypes **6* [including 830_831insA (Glu277fsX8)] (0.1%), **11* [including 1088C > T (Thr363Met)] (0.2%), **16B* [including 554C > G (Thr185Ser)] (1.4%), and **18B* [including 878T > C (Leu293Pro)] (2.8%) were identified, but **1B* (−392A > G) was not found. These findings indicate that ethnic-specific *CYP3A4* haplotypes must be taken into consideration in pharmacogenetic studies.

Irinotecan, an anticancer prodrug, is used for treatment of various cancers including lung and colon, and metabolized by CYP3A4 to produce inactive compounds such as APC (a major CYP3A4-mediated product) and NPC (a minor product) [6, 7]. An active metabolite SN-38 (a topoisomerase I inhibitor) is produced from the parent compound by carboxylesterases (CES) [28] and subsequently glucuronidated by UDP-glucuronosyltransferase 1As (UGT1As) to form inactive compound SN-38G [12] (Fig. 1). The parent compound and its metabolites are mainly excreted into the bile [29], where several ABC transporters, such as P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance-associated protein 2 (MRP2) are involved in excretion [30]. The dose-limiting toxicities of irinotecan are severe diarrhea and neutropenia, and high plasma concentrations of SN-38 and/or its accumulation in tissues are thought to cause these toxicities [3, 30]. Recent extensive pharmacogenetic studies on irinotecan, mostly focusing on the *UGT1A1* genotypes, have revealed important roles for *UGT1A1*28* and **6* in reduced in vivo UGT activity and enhanced toxicities [1, 8, 9, 11, 13, 22, 26]. On the other hand, CYP3A4 can modulate irinotecan pharmacokinetics (PK). Co-administration of ketoconazole, a CYP3A4 inhibitor and also a potent UGT1A1 inhibitor [34], with irinotecan resulted in a decreased value of the area under the concentration–time curve (AUC) for APC and also increased AUC for SN-38 [14]; and vice versa, co-administration of St. John's Wort,

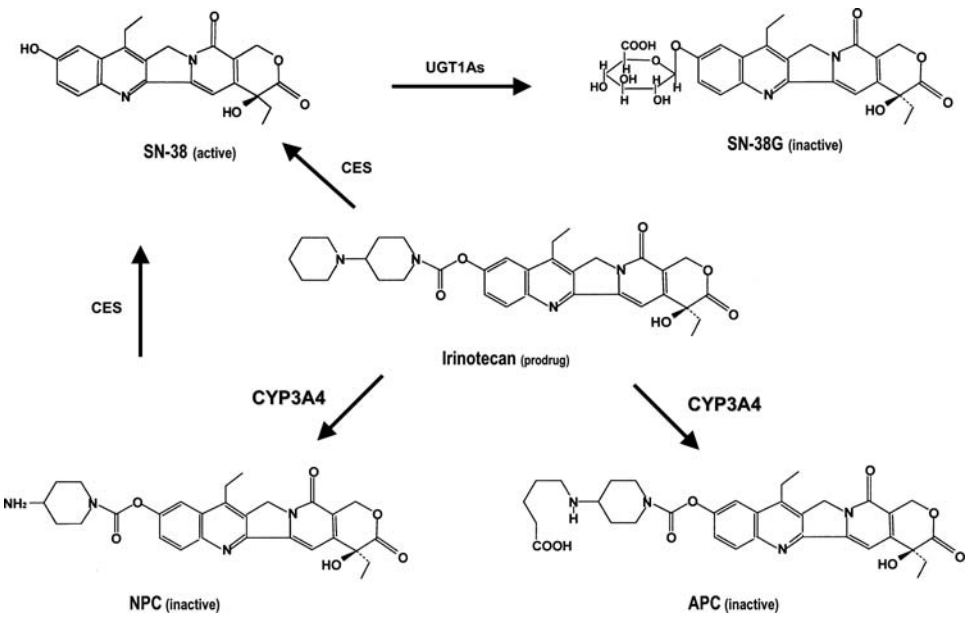


Fig. 1 Irintoecan metabolism in human liver. CYP3A4 mediates oxidation of irinotecan to produce inactive compounds, such as APC (a major CYP3A4-mediated product) and NPC (a minor product)

a CYP3A4 inducer, decreased the AUC of SN-38 [19]. A close association was also reported between in vivo CYP3A4 phenotypes and irinotecan clearance [21]. To date, however, no clinical impact by *CYP3A4* polymorphisms, such as **1B* (−392A > G) and **3* [1334T > C (Met445Thr)], has been demonstrated on irinotecan PK in Caucasians [20]. We previously found that **16* [554C > G (Thr185Ser)] caused decreased in vitro CYP3A4 activities [23]. Furthermore, a significant association of **16B* [harboring 554C > G (Thr185Ser)] was demonstrated with decreased AUC ratios of metabolite/paclitaxel, an in vivo parameter of CYP3A4 activity, in paclitaxel-administered Japanese patients [24].

In this study, to determine the clinical impact of the *CYP3A4* polymorphisms on irinotecan therapy, we identified the *CYP3A4* diplotypes of 177 Japanese cancer patients who received irinotecan and analyzed associations of the *CYP3A4* genotypes with irinotecan PK and toxicities.

Materials and methods

Patients and irinotecan treatment

One hundred seventy-seven patients with cancers who started irinotecan-containing therapy from 2002 to 2004 at two National Cancer Center Hospitals (Tokyo and Kashiwa, Japan) were enrolled for this pharmacogenetic study on irinotecan. This study was approved by the ethics committees of the National Cancer Center and the National Institute of Health Sciences, and written informed consent was obtained from all participants. No participant received irinotecan previously, and other eligibility criteria included: bilirubin < 2 mg/dl, aspartate aminotransferase (GOT) < 105 IU/l, alanine aminotransferase (GPT) < 120 IU/l, creatinine < 1.5 mg/dl, white blood cell count > 3000/μl, performance status of 0–2, and an interval of at least 4 weeks after the last session of chemotherapy (2 weeks after the last session of radiotherapy). Exclusion criteria were diarrhea, active infection, intestinal paralysis or obstruction, and interstitial pneumonitis. Irinotecan was administered as a single agent or in combination chemotherapy at the discretion of attending physicians. Doses and schedules were applied according to the approved treatment recommendations in Japan: intravenous 90-min infusion at a dose of 100 mg/m^2 weekly or 150 mg/m^2 biweekly for irinotecan-monotherapy, and 60 mg/m^2 weekly for combination therapy with cisplatin. Profiles of the patients and irinotecan regimens are summarized in Table 1.

Genotyping of *UGT1A1* and *CYP3A4*

DNA was extracted from pretreatment whole-blood samples taken from 177 patients who received irinotecan. Data on *UGT1A1* genetic polymorphisms obtained from the same set of DNA samples have been published elsewhere [22]. The *CYP3A4* genotypes for 88 patients were previously determined [4]. Additional *CYP3A4* genotyping for the remaining 89 patients was conducted using the pyrosequencing method described previously [24], and the *CYP3A4* diplotypes/haplotypes [4] were inferred using an expectation-maximization-based program, LDSUPPORT [15].

Pharmacokinetics and toxicities

Pharmacokinetic analysis for irinotecan in 176 patients (data on one patient was unavailable) was performed as

Table 1 Profiles of Japanese cancer patients in this study

			No. of patients
Patients for genotyping			177
(Male/female)			(135/42)
Age			
Mean/range	60.5/26–78		
Performance status	0/1/2		84/89/4
Combination therapy, tumor type and initial dose of irinotecan[a]			
Irinotecan monotherapy	Lung	100 (60–100)/w	21
	Colon	150 (120–150)/2w	28
	Others	100 (100–150)/w	7
With platinum-containing drug[b]	Lung	60 (50–90)/w	58
	Stomach	70/2w	9
	Others	60/w	5
With 5-fluorouracil (5-FU)/leucovorin (LV)[c] or tegafur/gimeracil/oteracil potassium[d]	Colon	100 (90–180)/w or 150/2w	34
	Others	90/w or 100/w	2
With mitomycin C (MMC)[e]	Stomach	150/2w	10
	Colon	150/2w	1
With amrubicin[f]	Lung	60/w	2

[a] The median value and range in the parentheses are shown. "/w" and "/2w" represent weekly and biweekly, respectively

[b] Mostly, cisplatin (60 or 80 mg/m^2) was administered after irinotecan treatment

[c] LV (10 mg/m^2) was administered right after irinotecan treatment and then followed by 5-FU treatment. (500 mg/m^2 injection); or LV (200 mg/m^2) was administered simultaneously with irinotecan and followed by 5-FU treatment (400 mg/m^2 bolus injection and 2.0–2.4 g/m^2 infusion)

[d] Tegafur (80 mg/m^2 per day)/gimeracil/oteracil potassium was administered twice (before irinotecan treatment and on the next day)

[e] MMC (5 mg/m^2) was administered just before irinotecan treatment

[f] Amrubicin (30 or 35 mg/m^2) was administered 24 h after irinotecan treatment

previously described [26]. Briefly, heparinized blood was collected before administration of irinotecan, and 0, 0.3, 1, 2, 4, 8, and 24 h after termination of the first infusion of irinotecan. Plasma concentrations of irinotecan and APC were determined by HPLC [25], and AUC_{inf} and other PK parameters were calculated using the trapezoidal method of the 202 non-compartmental model for a constant infusion in WinNonlin ver. 4.01 (Pharsight Corporation, Mountain View, CA, USA). As for the co-administered anti-cancer and other drugs which were administered within 1 week before irinotecan-treatment, no drugs significantly affected the PK parameters related to CYP3A4 activity. Information on foods and drinks taken by the patients which might induce or inhibit CYP3A4 activity was not available.

A complete medical history and data on physical examinations were recorded prior to irinotecan therapy. Complete blood cell counts with differentials and platelet counts, as well as blood chemistry, were measured once a week during the first 2 months of irinotecan treatment. Toxicities were graded according to the Common Toxicity Criteria of National Cancer Institute version 2. Association of genetic factors with irinotecan toxicities was analyzed primarily in patients who received irinotecan as a single agent.

Statistical analysis

Statistical analysis on the differences in PK parameters between sexes and among *CYP3A4* genotypes was performed using the Mann–Whitney test or Kruskal–Wallis test, and associations of *CYP3A4* genotypes with the irinotecan toxicities were assessed by the Chi-square test, using Prism version 4.0 (GraphPad Prism Software Inc. San Diego, CA, USA). $P = 0.05$ (two-tailed) was set as a significant level of difference. Multivariate analysis for the log-transformed AUC ratio (APC/irinotecan) was performed using age, sex, body surface area, dosage of irinotecan, history of smoking or drinking, performance status, co-administered drugs, serum biochemistry parameters at baseline, and genetic factors (including *CYP3A4* haplotypes and the *UGT1A1*6 or *28* haplotype obtained in our previous study [22]) as independent variables. Multivariate analysis on toxicities (grade 3 diarrhea or nadir of absolute neutrophil counts) was conducted for the patients who received irinotecan monotherapy, where the variables included dosing interval and the absolute neutrophil count at baseline, in addition to the other patient background and genetic factors described above. The variables in the final

models for both AUC ratio and toxicities were chosen by the forward and backward stepwise procedure at the significance level of 0.1 using JMP version 6.0.0 software (SAS Institute, Inc., Cary, NC, USA).

Results

Sex difference in PK parameters

Since hepatic CYP3A4 levels were reported to be significantly higher in females than in males [24, 32], we first analyzed the sex differences in the major PK parameters for irinotecan and APC, a major CYP3A4 metabolite (Table 2). As for irinotecan, lower total clearance and MRT, and higher AUC/dose were observed in females, but the differences (3, 5 and 3%, respectively) were not significant. A small but significant increase in C_{max}/dose for irinotecan was observed in females. This is attributable to the smaller distribution volume of females. On the other hand, the median values of AUC/dose and C_{max}/dose for APC of the females were significantly higher than those of the males (1.29- and 1.33-fold, respectively). The AUC ratio (APC/irinotecan), a parameter of in vivo CYP3A4 activity, was significantly higher (1.28-fold) in females than in males. These findings suggest that these differences may reflect the higher CYP3A4 activity in the females.

CYP3A4 genotypes

CYP3A4 diplotypes/haplotypes in 177 Japanese cancer patients were determined according to the previous definition [4]. The *CYP3A4* haplotypes found in this population were **1A* (wild type), **1G* (IVS10 + 12G > A alone), **16B* [554C > G (Thr185Ser) and IVS10 + 12G > A], and **18B* [878T > C (Leu293Pro) and IVS10 + 12G > A]. In the current study, neither **6* [830_831insA (Glu277fsX8)] nor **11* [1088C > T (Thr363Met)] were found. The frequencies of **1G*, **16B*, and **18B* were 0.215, 0.014, and 0.020 (Table 3), and they were comparable to those obtained in previous reports [4, 24]. Note that the haplotypes **16B* and **18B* were detected only in male patients.

Associations of *CYP3A4* genotypes with PK parameters

Considering the significant sex difference in APC levels, associations between the *CYP3A4* genotypes and PK parameters were analyzed for each sex separately. In male patients, no significant differences among the *CYP3A4* genotypes were observed for total clearance and MRT of irinotecan (Fig. 2a, b). In females, a slightly but significantly lower (10%) median value for MRT of irinotecan was observed in patients bearing **1G* compared with those carrying the wild type (**1A*/**1A*) ($P = 0.022$, Mann–Whitney test) (Fig. 2b), whereas no significant **1G*-dependency was observed for total clearance (Fig. 2a). No significant

Table 3 Frequencies of *CYP3A4* haplotypes (A) and diplotypes (B) for Japanese cancer patients in this study

(A) Haplotype group[a]	No. of chromosomes ($N = 354$)	Frequency
**1A*	266	0.751
**1G*	76	0.215
**16B*	5	0.014
**18B*	7	0.020
(B) Diplotype	No. of patients ($N = 177$)	Frequency
1A*/1A*	100	0.565
1G*/1A*	55	0.311
1G*/1G*	10	0.056
16B*/1A*	4	0.023
16B*/1G*	1	0.006
18B*/1A*	7	0.040

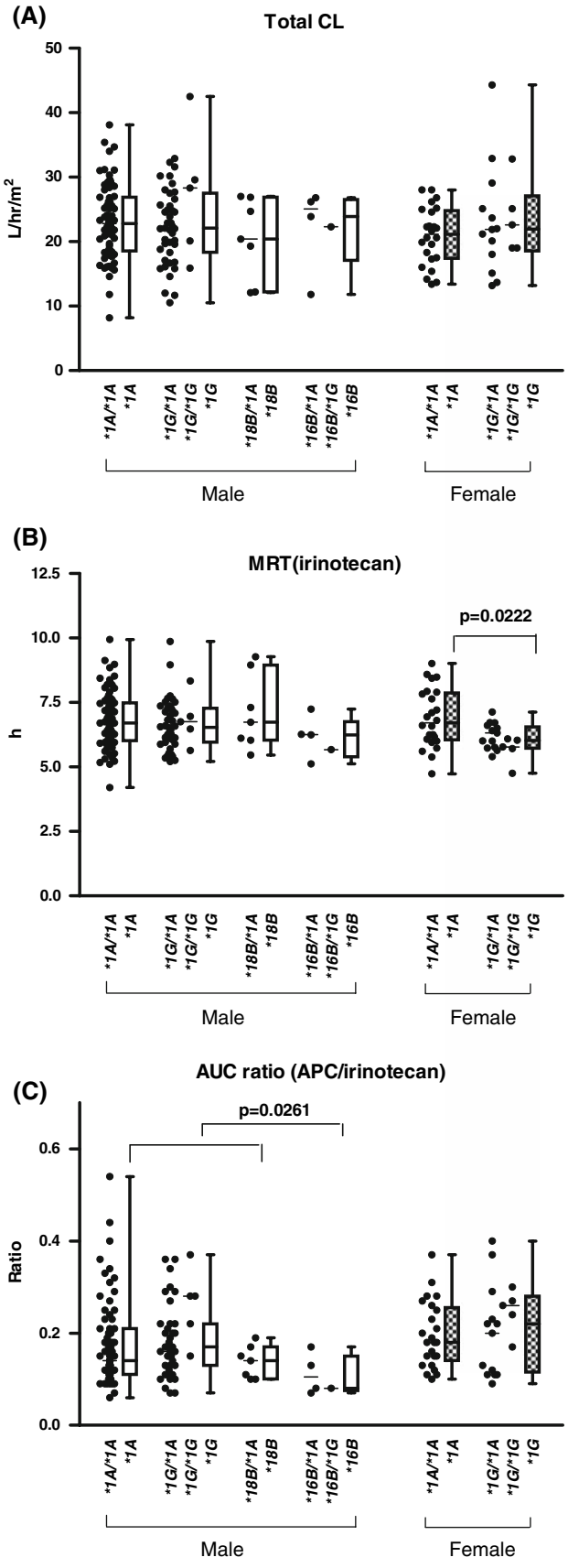
[a] Groups based on tagging SNPs of major haplotypes previously defined [4]; **1A* wild type, **1G* IVS10 + 12G > A; **16B* 554C > G (Thr185Ser) and IVS10 + 12G > A; **18B* 878T > C (Leu293Pro) and IVS10 + 12G > A

Table 2 Pharmacokinetic parameters for irinotecan-administered Japanese patients and sex differences

Parameters	Male ($N = 134$) Median (25–75‰)	Female ($N = 42$) Median (25–75‰)	P value[a]
Irinotecan			
Total CL (l/h per m^2)	22.6 (18.5–26.9)	21.8 (17.8–25.1)	0.242
AUC/dose (10^{-3} h m^2 per l)	44.4 (37.3–54.1)	45.8 (39.8–55.8)	0.242
C_{max}/dose (10^{-3} m^2 per l)	10.0 (8.96–11.3)	11.4 (10.4–12.4)	0.0003
MRT (h)	6.61 (6.01–7.40)	6.29 (5.78–7.12)	0.202
APC			
AUC/dose (10 h m^2 per l)	6.72 (5.23–9.49)	8.66 (6.57–13.1)	0.0071
C_{max}/dose (10^{-3} m^2 per l)	0.560 (0.430–0.805)	0.745 (0.610–1.14)	0.0007
AUC ratio (APC/irinotecan)	0.151 (0.114–0.210)	0.194 (0.132–0.266)	0.0179

CL clearance; *MRT* mean residence time

[a] Mann–Whitney test



◀ **Fig. 2** Association of *CYP3A4* genotypes with irinotecan pharmacokinetics in Japanese cancer patients. The values of mean residence time (MRT) of irinotecan in female patients were significantly lower in those with **1G* than those with the wild-type (**1A/*1A*) ($P = 0.0222$, Mann–Whitney test). The levels of the AUC ratio (APC/irinotecan), a parameter of CYP3A4 activity, in male patients were significantly lower in those with **16B* than those without **16B* ($P = 0.0261$, Mann–Whitney test)

differences in C_{max}/dose for irinotecan among the genotypes were observed in both males and females (data not shown). Regarding the AUC ratio (APC/irinotecan) in males, a significantly lower median value (50%) was observed in patients with **16B* than patients without **16B* (i.e., *non-*16B* patients) ($P = 0.0261$, Mann–Whitney test) (Fig. 2c). In contrast, no significant changes in the AUC ratio (APC/irinotecan) were detected in the male **18B* heterozygotes. In both males and females, a higher median AUC ratio (20%), without statistical significance, was observed in **1G*-bearing patients (**1G/*1A* and **1G/*1G*) than wild-type patients (**1A/*1A*). As for C_{max}/dose of APC, similar trends were observed (without statistical significance): 35% decrease in the median value for **16B* compared with *non-*16B*; 10 and 20% increases in males and females, respectively, for **1G* compared with the wild type (data not shown).

Multivariate analysis of PK parameters

To further clarify contributions of the *CYP3A4* polymorphisms to APC generation, multivariate analysis was conducted on the AUC ratio (APC/irinotecan) data, where variables included patient backgrounds, irinotecan regimens, and *CYP3A4* (**1G, *16B* and **18B*) and *UGT1A1 (*6 or *28)* haplotypes. Significant contributions of *CYP3A4*16B* (coefficient ± SE = -0.18 ± 0.077, $P = 0.021$) and **1G* (0.047 ± 0.021, $P = 0.029$) to the AUC ratio (APC/irinotecan) were confirmed, in addition to the contributions of two patient background factors, sex (female) and hepatic function (serum GOT and ALP) (Table 4). No significant associations were observed between the *CYP3A4* polymorphisms and total clearance or MRT of irinotecan (data not shown).

Associations of *CYP3A4* genotypes with toxicities

Severe irinotecan toxicities, grade 3 diarrhea and grade 3 or 4 neutropenia, were monitored in 176 patients during 2 months after starting irinotecan therapy. Since incidences of severe toxicities depended on the irinotecan regimens used and a higher incidence of severe neutropenia with comedication was evident [22], associations of the *CYP3A4*

Table 4 Multivariate analysis of AUC ratio (APC/irinotecan)

Variable	Coefficient	SE	*P* value
Female	0.040	0.016	0.0132
Serum GOT and ALP[a]	0.110	0.021	<0.0001
Serum creatinine[b]	0.132	0.071	0.0651
*CYP3A4*16B*	−0.180	0.077	0.0213
*CYP3A4*1G*	0.047	0.021	0.0291

The values after logarithmic conversion were used

R^2 0.225; Intercept −0.794; *N* 176

[a] Grade 1 or greater scores in both serum GOT and ALP before irinotecan treatment

[b] The absolute value (mg/dl) before irinotecan treatment

haplotypes with toxicities were evaluated in patients who received irinotecan monotherapy. Because there was no sex difference in the incidences of severe toxicities, the patients with irinotecan monotherapy were not stratified by sex. Furthermore, significant contributions of *UGT1A1*6* and **28* to neutropenia were previously demonstrated [22]. Therefore, the incidence of severe neutropenia was also evaluated among the wild-type patients without *UGT1A1*6 or *28* (*UGT* −/−). No significant differences in the incidences of severe diarrhea and neutropenia were observed among the *CYP3A4* diplotypes of all or *UGT* −/− patients with irinotecan monotherapy (Table 5). It must be noted that the **16B*-bearing patient (*N* = 1) treated with irinotecan monotherapy did not experience either toxicity. Similarly, for **1G* and **18B*, no statistically significant change in the neutropenia or diarrhea incidence was observed. Multivariate analysis also revealed no significant contribution of the *CYP3A4* polymorphisms to severe diarrhea (logistic model) or absolute neutrophil count nadir (data not shown).

Table 5 Association of *CYP3A4* genotypes with severe toxicities in irinotecan monotherapy

Diplotype	Diarrhea[a]/total (%)	Neutropenia[b]/total (%)	
	All	All	UGT-/-[c]
**1A/*1A*	3/27 (11.1)	5/27 (18.5)	2/11 (18.2)
**1G/*1A*	2/20 (10.0)	5/20 (25.0)	1/9 (11.1)
**1G/*1G*	0/3 (0.0)	2/3 (66.7)	0/0 (–)
**16B/*1A*	0/1 (0.0)	0/1 (0.0)	0/0 (–)
**18B/*1A*	1/4 (25.0)	2/4 (50.0)	0/1 (0.0)
P value[d]	0.8571	0.289	

[a] Grade 3

[b] Grade 3 or 4

[c] Wild type without *UGT1A1 *6 or *28*

[d] Chi-square test

Discussion

In the current study, the higher in vivo CYP3A4 activity in females than in males [24, 32] was suggested from the CYP3A4-mediated APC formation. Since correlations between in vivo CYP3A4 activity and irinotecan PK parameters have been reported [14, 19, 21], clinical impact of *CYP3A4* polymorphisms on irinotecan PK has been presumed. In this study, we demonstrated for the first time a role of *CYP3A4*16B* [554C > G (Thr185Ser) and IVS10 + 12G > A] in reduced APC generation (Fig. 2; Table 4). This finding is concordant with the findings of our previous studies showing a reduced in vitro activity of CYP3A4 by **16* [23] and altered AUC ratios of metabolite/paclitaxel in paclitaxel-administered Japanese patients bearing **16B* [24]. These findings indicate that *CYP3A4*16* could modulate pharmacokinetics of other drugs which are metabolized by CYP3A4. On the contrary, **18B* [878T > C (Leu293Pro) and IVS10 + 12G > A] did not alter the AUC ratios (APC/irinotecan) in irinotecan-administered patients. This also coincides with our previous finding that showed no clinical impact of **18B* on the metabolite/paclitaxel AUC ratio [24].

In the current study, an increasing trend in the AUC ratios (APC/irinotecan) by **1G* (IVS10 + 12G > A) was detected in both males and females, although their increases were small (20% in the median values). In accordance with this tendency, significant reduction in MRT of irinotecan by **1G* was observed in females, whereas this was not significant in males. At present, the reason of this sex-difference in MRT is not clear. Our previous haplotype analysis of the *CYP3A4* and *CYP3A5* regions revealed that *CYP3A4*1G* is mostly linked to *CYP3A5*1* but rarely to *CYP3A5*3* [3] which is a defective allele [10, 16, 17, 33]. Therefore, there is a possibility that *CYP3A5* polymorphisms rather than *CYP3A4*1G* contribute to irinotecan PK. However, this speculation is unlikely because CYP3A5 produces only a very minor metabolite of irinotecan, a de-ethylated product [27]. Since the effect of **1G* was relatively small and was not shown in case of paclitaxel [23], the clinical importance of **1G* should be further evaluated in pharmacogenetic studies on other drugs.

Contrary to the clear reduction in APC production, changes in the PK parameters for the parent compound, i.e., total clearance and Cmax of irinotecan, were not affected by the *CYP3A4* haplotypes. Furthermore, multivariate analysis revealed no associations of the *CYP3A4* haplotypes with the AUC ratio of (SN-38 + SN-38G)/irinotecan, an in vivo parameter for CES activity, and with the AUC ratio of SN-38 (SN-38/irinotecan) (data not shown). We previously observed that the total clearance of irinotecan was affected by other non-genetic factors, such as age, smoking, hepatic and renal functions, and co-administered drugs

(unpublished data), and that the plasma level of SN-38 was largely influenced by *UGT1A1*6* and **28* [22]. Therefore, it is likely that the contribution of CYP3A4 to irinotecan clearance is rather small as compared with other genetic and non-genetic factors.

In accordance with the above observations, no significant associations were observed between the *CYP3A4* haplotypes and severe toxicities (grade 3 diarrhea and grade 3 or 4 neutropenia) in the patients with irinotecan monotherapy (Table 5). Similarly, we observed no significant effect of the *CYP3A4* haplotypes on incidence of the severe toxicities in the patients treated with both irinotecan and cisplatin (data not shown), although the numbers of patients bearing **16B* and **18B* were small. Taken together, the current study indicates that the influence of the *CYP3A4* genotypes on the activation pathway of irinotecan (generation of SN-38) might be small.

In conclusion, the current study suggested that *CYP3A4*16B* was associated with decreased metabolism of irinotecan to APC. However, impact of the *CYP3A4* haplotypes on total clearance of irinotecan and severe toxicities was not significant.

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