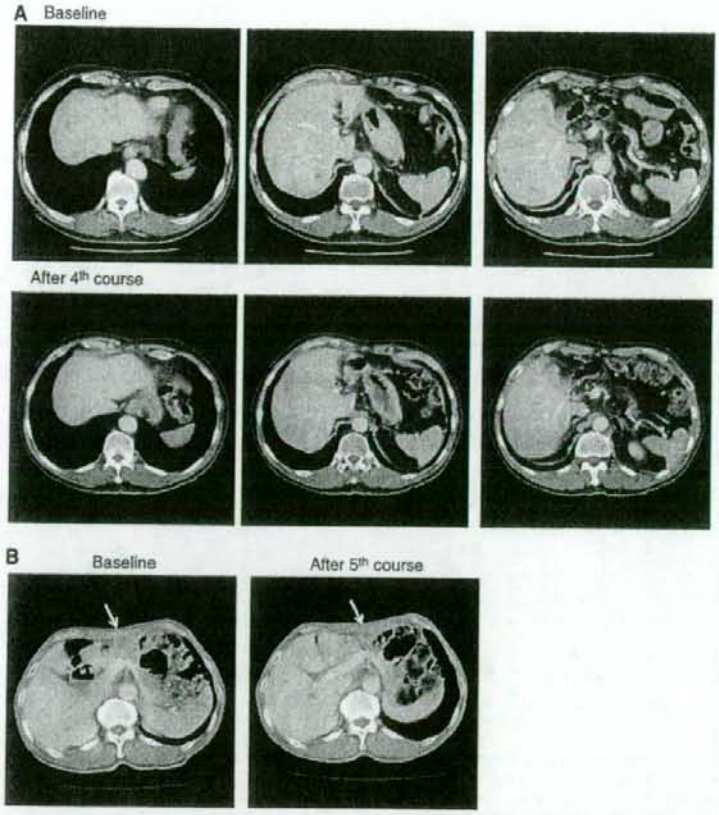


**Figure 3** Serial CT scans. (**A**) A 60-year-old male with pancreatic cancer who was treated with NK105 at a dose level of 150 mg m$^{-2}$. Baseline scan (upper panels) showing multiple metastasis in the liver. Partial response, characterized by a more than 90% decrease in the size of the liver metastasis (lower panels) compared with the baseline scan. The antitumour response was maintained for nearly 1 year. (**B**) A 64-year-old male with stomach cancer who was treated with NK105 at a dose level of 150 mg m$^{-2}$. Baseline scan (left panel) showing a peritoneal metastasis and liver metastasis. About 40% reduction (right panel) was observed in peritoneal metastasis, but not in the liver metastasis after fifth course.

**Table 4** Pharmacokinetic parameters

| | Dose (mg m$^{-2}$) | *n* | $C_{max}$ (μg ml$^{-1}$) | $AUC_{0-inf.}$ (μg h$^{-1}$ ml$^{-1}$) | $t_{1/2}$ (h) | $CL_{tot}$ (ml h$^{-1}$ m$^{-2}$) | $V_{ss}$ (ml m$^{-2}$) | UE (%) | $CL_r$ (ml h m$^{-2}$) |
|---|---|---|---|---|---|---|---|---|---|
| NK105 | 150 | 7 | 40.1699 ±5.5334 | 369.8 ±35.2 | 10.6 ±1.3 | 408.6 ±37.3 | 4527.1 ±639.5 | 5.3 ±1.5 | 21.6 ±6.5 |
| PTX | 210 | 5 | 6.744 ±2.733 | 23.18 ±10.66 | 13.3 ±1.5 | 10740 ±4860 | 58900 ±24700 | 9.45 ±3.76 | 1020 ±648 |
| XYOTAX[a] | 233 | 4 | NA | 1583 ±572 | 120 ±28 | 276 ±63 | 6200 ±2100 | NA | NA |
| Abraxane | 300 | 5 | 13.52 ±0.95 | 17.61 ±3.70 | 14.6 ±2.04 | 17700 ±3894 | 370000 ±85100 | NA | NA |
| Genoxol-PM | 300 | 3 | 3.107 ±1.476 | 11.58 ±4.28 | 11.4 ±2.4 | 29300 ±13800 | NA | NA | NA |

[a]Conjugated taxanes.

system. When compared with conventional PTX at a dose of 210 mg m$^{-2}$ (conventional dose for a 3-week regimen in Japanese patients), NK105 at a dose of 150 mg m$^{-2}$ (recommended phase II dose) exhibited more than 15-fold larger plasma AUC and a 26-fold lower $CL_{tot}$. The larger plasma AUC is consistent with the stability of the micelle formulation in plasma. The $V_{ss}$ of NK105 was 13-fold lower than that of conventional PTX. This suggests that PTX may have a relatively lower distribution in normal tissue, including normal neural tissue, following NK105 administration. Regarding the drug distribution in tumours, nanoparticle drug carriers have been known to preferentially accumulate in tumour tissues utilising the EPR effect (Matsumura and Maeda, 1986;

Maeda *et al*, 2000; Duncan, 2003). We speculate that NK105 accumulates more in tumour tissues than free PTX, since NK105 is very stable in the circulation and exhibits a markedly higher plasma AUC than free PTX. Moreover, a polymeric micelle carrier system for a drug has the potential to enable the sustained release of the drug inside a tumour following the accumulation of micelles in the tumour tissue (Hamaguchi *et al*, 2005; Uchino *et al*, 2005; Koizumi *et al*, 2006). Regarding NK105 in particular, this sustained release may begin at a PTX-equivalent dose of $<1\,\mu g\,ml^{-1}$ (data not shown). Consequently, the released PTX is distributed throughout the tumour tissue where it kills the cancer cells directly.

In the present study, NK105 appeared to exhibit characteristic pharmacokinetics different from those of other PTX formulations including conventional PTX, Abraxane, Genexol-PM, and Xyotax. For example, previous clinical PK data at each phase II recommended dose shown that plasma AUC and $C_{max}$ were 11.58 and 3.1 in Genexol-PM (Table 4). The antitumour activities seen in two patients with intractable cancers are encouraging. In addition, we recently demonstrated in preclinical study that combined NK105 chemotherapy with radiation exerts a significantly more potent antitumour activity, compared with combined PTX therapy and radiation (Negishi *et al*, 2006). This data on NK105 justifies its continued clinical evaluation.

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# Irinotecan pharmacokinetics/pharmacodynamics and *UGT1A* genetic polymorphisms in Japanese: roles of *UGT1A1*6* and **28*

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***Objectives*** **SN-38, an active metabolite of irinotecan, is detoxified by glucuronidation with UGT1A isoforms, 1A1, 1A7, 1A9, and 1A10. The pharmacogenetic information on *UGT1A* haplotypes covering all these isoforms is important for the individualized therapy of irinotecan. Associations between *UGT1A* haplotypes and pharmacokinetics/pharmacodynamics of irinotecan were investigated to identify pharmacogenetic markers.**

***Methods*** **Associations between *UGT1A* haplotypes and the area under concentration curve ratio (SN-38 glucuronide/SN-38) or toxicities were analyzed in 177 Japanese cancer patients treated with irinotecan as a single agent or in combination chemotherapy. For association analysis, diplotypes of *UGT1A* gene segments [(*1A1*, *1A7*, *1A9*, *1A10*), and Block C (common exons 2–5)] and combinatorial haplotypes (*1A9-1A7-1A1*) were used. The relationship between diplotypes and toxicities was investigated in 55 patients treated with irinotecan as a single agent.**

***Results*** **Among diplotypes of *UGT1A* genes, patients with the haplotypes harboring *UGT1A1*6* or **28* had significantly reduced area under concentration curve ratios, with the effects of *UGT1A1*6* or **28* being of a similar scale. A gene dose effect on the area under concentration curve ratio was observed for the number of haplotypes containing **28* or **6* (5.55, 3.62, and 2.07 for 0, 1, and 2 haplotypes, respectively, $P<0.0001$). In multivariate analysis, the homozygotes and double heterozygotes of **6* and **28* (**6/*6*, **28/*28* and **6/*28*) were significantly associated with severe neutropenia in 53 patients who received irinotecan monotherapy.**

***Conclusions*** **The haplotypes significantly associated with reduced area under concentration curve ratios and neutropenia contained *UGT1A1*6* or **28*, and both of them should be genotyped before irinotecan is given to Japanese and probably other Asian patients. *Pharmacogenetics and Genomics* 17:497–504 © 2007 Lippincott Williams & Wilkins.**

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## Introduction

Irinotecan, an anticancer prodrug, is widely applied for colorectal, lung, stomach, ovarian, and other various cancers. It is activated by carboxylesterases to SN-38 (7-ethyl-10-hydroxycamptothecin), which shows antitumor activity by inhibiting topoisomerase I [1,2]. SN-38 is subsequently glucuronidated by uridine diphosphate glucuronosyltransferases (*UGTs*) to form an inactive metabolite, SN-38 glucuronide (SN-38G) [3]. Dose-limiting toxicities of irinotecan are diarrhea and leukopenia [4], and reduced activity for SN-38G formation is closely related to severe toxicities [5]. Among UGT isoforms, UGT1A1 is abundant in both the liver and intestine and is thought to be mainly responsible for inactivation of SN-38 [3,6]. Genetic polymorphisms of *UGT1A1* result in reduced enzyme activity and increased toxicity by irinotecan. A significant association of *UGT 1A1*28*, a repeat polymorphism of the TATA box (-40_-39insTA) [3,7], with severe irinotecan-induced diarrhea/leukopenia was first reported in a retrospective study of Japanese cancer patients [8]. Subsequent pharmacogenetic studies in Caucasians have shown close associations of **28* with reduced glucuronidation of SN-38 and/or severe neutropenia/diarrhea [9–12]. These

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studies have clearly indicated that **28* is a good genetic marker for individualized irinotecan therapy. On the basis of these observations, the Food and Drug Administration of the United States has approved an amendment of the label for Camptosar (irinotecan HCl) and added a warning to consider a reduction in the starting dose of irinotecan for **28* homozygous patients (NDA 20-571/S-024/S-027/S-028).

There is significant racial difference in *UGT1A1* polymorphisms among Asians, Caucasians, and Africans [13]. Although the association of *UGT1A1*28* with toxicities by irinotecan was first described in Japanese patients, its frequency in Japanese is one-third of that in Caucasians. Another low-activity allele **6* [211G > A(G71R)], which is not detected in Caucasians or Africans, is as frequent as the **28* allele in Japanese. Moreover, the area under concentration curve (AUC) ratio of SN-38G to SN-38 was decreased in patients having **6* haplotypes [14].

In addition to UGT1A1, recent studies have suggested possible contributions to SN-38G formation by UGT1A7, 1A9, and 1A10 [15–17], which are expressed in the gastrointestinal tract, the liver and intestine, and extrahepatic tissues, respectively [18]. Altered activity resulted from genetic polymorphisms of these isoforms, including *1A7*3* [387T > G(N129K), 391C > A(R131K), 622T > C(W208R)], *1A9*22* (-126_-118$T_9$ > $T_{10}$), *1A9*5* [766G > A(D256N)], and *UGT1A10*3* [605C > T(T202I)], but clinical relevance of these polymorphisms is yet to be elucidated [16,19–24]. Moreover, close linkages among *1A9*, *1A7*, and *1A1* polymorphisms were found in Caucasians and Asians in an ethnic-specific manner [20,25–27]. Therefore, comprehensive investigation that covers these genes, along with linkages among the polymorphisms, is needed, in each ethnic population, to evaluate associations between the genetic polymorphisms and pharmacokinetics, as well as clinical outcomes of irinotecan therapy.

Recently, we have analyzed the segmental and block haplotypes of *1A8*, *1A10*, *1A9*, *1A7*, *1A6*, *1A4*, *1A3* and *1A1*, and the common exons 2–5 (Block C) in a Japanese population, including the 177 cancer patients treated with irinotecan, and showed close linkages between the haplotypes, that is, *1A9*22* and *1A7*1*, *1A7*3* and *1A1*6*, and *1A7*3* and *1A1*28* [28]. Preliminary results of *UGT1A1* pharmacogenetics on 85 of these cancer patients were reported previously [14]. In the current study, we investigated the pharmacogenetics of irinotecan, focusing on diplotypes of the *UGT1A* complex covering *1A1*, *1A7*, *1A9*, *1A10*, and Block C (exons 2–5) of 177 patients, so as to elucidate haplotypes or genetic markers associated with altered glucuronidation of SN-38 and toxicities.

## Methods

### Patients and treatment schedule

Patients with cancers who started chemotherapy with irinotecan at two National Cancer Center Hospitals (Tokyo and Kashiwa, Japan) were eligible if they had not received irinotecan previously. Other eligibility criteria included bilirubin $\leq 2$ mg/dl, aspartate aminotransferase (GOT) $\leq 105$ IU/l, alanine aminotransferase (GPT) $\leq 120$ IU/l, creatinine $\leq 1.5$ mg/dl, white blood cell count $\geq 3000/\mu l$, performance status of 0–2, and at least 4 weeks after the last chemotherapy (2 weeks for radiotherapy). Exclusion criteria were diarrhea, active infection, intestinal paralysis or obstruction, and interstitial pneumonitis. The ethics committees of the National Cancer Center and the National Institute of Health Sciences approved this study, and written informed consent was obtained from all participants.

Irinotecan was administered as a single agent or in combination chemotherapy at the discretion of attending physicians. Doses and schedules were according to approved usage in Japan; intravenous 90-min infusion at a dose of 100 mg/m$^2$ weekly or 150 mg/m$^2$ biweekly. In terms of combination chemotherapy, the dose of irinotecan was reduced according to clinical protocols.

### Genetic polymorphisms of UGT1As and pharmacokinetics

Detailed assay methods for genotypes of the *UGT1A* gene complex were reported previously [14,28]. In this study, we focused on the genetic variations in *UGT1A1*, *1A7*, *1A9*, and *1A10* and common exons 2–5, as they have been reported to contribute to the SN-38 glucuronidation. Haplotype analysis covering these regions was performed in our previous study [28], and haplotypes of each *UGT1A* segment [exon 1 for *1A1*, *1A7*, *1A9*, or *1A10*; and Block C (common exons 2–5)] are summarized in Fig. 1.

Pharmacokinetic analysis for irinotecan was performed as described previously [14]. Briefly, heparinized blood was collected before administration of irinotecan, as well as 0 and 20 min, and 1, 2, 4, 8, and 24 h after termination of the first infusion of irinotecan. Plasma concentrations of irinotecan, SN-38 and SN-38G were determined by the high-performance liquid chromatography [29], and AUC was calculated by the trapezoidal method using WinNonlin version 4.01 (Pharsight Corporation, Mountain View, California, USA). Associations between genotypes and the AUC ratio (AUC of SN-38G/AUC of SN-38) were evaluated in 176 patients.

### Monitoring and toxicities

A complete medical history and data on physical examinations were recorded before the 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.

**Fig. 1**

*UGT1A1*

| Region | Enhancer | Promoter | Exon 1 | | Frequency |
|---|---|---|---|---|---|
| Nucleotide change | −3279 T>G | −40_−39 insTA | 211 G>A | 686 C>A | |
| Amino acid change | | | G71R | P229Q | |
| Marker allele | *60 | *28 | *6 | *27 | |
| Haplotype *1 | | | | | 0.548 |
| Haplotype *6 | | | | | 0.167 |
| Haplotype *60 | | | | | 0.147 |
| Haplotype *28 *28b | | | | | 0.138 |
| Haplotype *28 *28c | | | | | |
| Haplotype *28 *28d | | | | | |

*UGT1A10*

| Region | Exon 1 | | | | Frequency |
|---|---|---|---|---|---|
| Nucleotide change | 4 G>A | 177 G>A | 200 A>G | 605 C>T | |
| Amino acid change | A2T | M59I | E67G | T202I | |
| Marker allele | *2T | *2 | *67G | *3 | |
| Haplotype *1 | | | | | 0.981 |
| Haplotype *2 | | | | | 0.006 |
| Haplotype *2T | | | | | 0.003 |
| Haplotype *3 | | | | | 0.010 |
| Haplotype *67G | | | | | 0.000 |

*UGT1A7*

| Region | Exon 1 | | | | Frequency |
|---|---|---|---|---|---|
| Nucleotide change | 387 T>G | 391 C>A | 392 G>A | 622 T>C | |
| Amino acid change | N129K | R131K | | W208R | |
| Marker allele | *2,*3 | *2,*3 | *2,*3 | *3,*4 | |
| Haplotype *1 | | | | | 0.630 |
| Haplotype *2 | | | | | 0.147 |
| Haplotype *3 | | | | | 0.223 |

*Block C*

| Region | Exon.4 | Exon.5 | | 3'-UTR | | | Frequency |
|---|---|---|---|---|---|---|---|
| Nucleotide change | 1091 C>T | 1456 T>G | 1598 A>C | *211(7813) C>T | *399 (8941) C>G | *440(9402) C>G | |
| Amino acid change | P364L | Y486D | H533P | | | | |
| Marker allele | *364L | *7 | *533P | *1B | *1B | *1B | |
| Haplotypes *1A | | | | | | | 0.864 |
| Haplotypes *1B *1b–*1j | | | | | | | 0.127 |
| Haplotypes *1B *533P | | | | | | | |
| Haplotypes *7 | | | | | | | 0.003 |
| Haplotypes *364L | | | | | | | 0.006 |

*UGT1A9*

| Region | Promoter | | Exon1 | | | Frequency |
|---|---|---|---|---|---|---|
| Nucleotide change | −126_−118 T9>T10 | −126_−118 T9>T11 | 422 C>G | 726 T>G | 768 G>A | |
| Amino acid change | | | S141C | Y242X | D256N | |
| Marker allele | *22 | *T11 | *141C | *4 | *5 | |
| Haplotype *1 | | | | | | 0.347 |
| Haplotype *22 | | | | | | 0.644 |
| Haplotype *141C | | | | | | 0.000 |
| Haplotype *4 | | | | | | 0.000 |
| Haplotype *5 | | | | | | 0.006 |
| Haplotype *T11 | | | | | | 0.003 |

Haplotypes of *UGT1A* gene segments (*UGT1A1*, *1A7*, *1A9*, *1A10*, and Block C) in 177 Japanese cancer patients. The tagging variations and haplotypes are shown. Variant alleles are indicated in grey. Definition of Block C haplotypes in our previous paper ([14]) (corresponding to Block 2) were slightly modified.

### Statistical analysis

Statistical analysis on the differences in the AUC ratios (SN-38G/SN-38) among *UGT1A* genotypes was performed using the Kruskal–Wallis test, followed by nonparametric Dunnett's multiple comparison test, or with Wilcoxon test. Analysis of a gene–dose effect of each haplotype was performed using the Jonckheere–Terpestra test in the SAS system, version 5.0 (SAS Institute, Cary, North Carolina, USA). Relationship of *UGT1A* genetic polymorphisms to the toxicities of irinotecan was assessed by the $\chi^2$ test via the use of using Prism version 4.0 (GraphPad Prism Software, San Diego, California, USA). The *P*-value of 0.05 (two-tailed) was set as a significant level, and the multiplicity adjustment was conducted for pharmacokinetics data with the false discovery rate [30].

To identify factors associated with the log-transformed AUC ratio of SN-38G/SN-38, multiple regression analysis was performed using age, sex, body surface area, dosage of irinotecan, history of smoking or drinking, performance status, coadministered drugs, serum biochemistry parameters at baseline, and *1A9-1A7-1A1* and Block C haplotypes (five or more chromosome numbers) or '*1A1*6* or *28'. For multiple regression analysis of neutropenia, variables included the absolute neutrophil count at baseline and the dosing interval, in addition to

the other patient background factors described above. The multivariate analyses were performed by using JMP version 6.0.0 software (SAS Institute). The variables in the final models for both AUC ratio and neutropenia were chosen by forward and backward stepwise procedures at significance levels of 0.25 and 0.05, respectively.

## Results

### Patients and UGT1A haplotypes

Patient demographics and information on the treatment are summarized in Table 1. In addition to UGT1A1, UGT1A7, 1A9, and 1A10 were also reported to glucuronidate SN-38 [15–17]. In our previous study, haplotype analysis covering the *1A9* to *1A1* (5′–3′) gene segments was conducted, and the combinatorial diplotypes (*1A9-1A7-1A1*) of the patients were determined. It must be noted that close linkages between *1A9*22* and *1A7*1*, between *1A7*2* and *1A1*60*, and between *1A7*3* and *1A1*6* or *1A1*28* were observed as described previously [28]. To clarify the linkages between these segmental haplotypes (*1A9*, *1A7*, and *1A1*), we grouped the combinatorial (*1A9-1A7-1A1*) haplotypes into four categories (A–D) based on the *1A1* haplotypes (**1*, **6*, **60*, and **28*). Each group was further divided into the subgroups based on the previously defined Block 9/6 (including *1A9*, *1A7*, and *1A6*) haplotypes (Table 2). The frequency of Group B haplotypes (*B1-B4*) harboring *1A1*6* was 0.167 and higher than that of Group D haplotypes (*D1-D6*) with **28* (0.138) in this population.

### Association of 1A9-1A7-1A1 diplotypes to SN-38G formation

When relationship between the *UGT1A* diplotypes (*1A9-1A7-1A1*) and the SN-38G/SN-38 AUC ratio was analyzed in the 176 cancer patients the AUC ratio for the diplotypes of *B2/B2*, *D2/A1*, and *D1/B2* was statistically significantly lower than the *A1/A1* diplotype (Fig. 2). These diplotypes harbored *1A1*6*, **28* or both. Significant gene–dose effects of *B2* (among *A1/A1*, *B2/A1*, and *B2/B2*) and *C3* (among *A1/A1*, *C3/A1*, and *C3/C3*) were also observed (Fig. 2). As no significant differences in AUC ratios were observed between *D1/A1* and *D2/A1*, *D1/C3* and *D2/C3*, and *D1/B2* and *D2/B2*, the haplotype combination *1A9*1-1A7*3* or *1A9*22-1A7*1* was not influential on the AUC ratio.

As the effect of diplotypes harboring *UGT1A1* polymorphism was prominent, we grouped the whole gene (*1A9-1A7-1A1*) diplotypes according to the *1A1* diplotypes (the upper part of Fig. 2). Patients with **6* or **28* (except for **28/*28*) haplotypes had significantly lower AUC ratios than the wild-type (**1/*1*), and significant gene–dose effects were observed for **28* (among **1/*1*, **28/*1*, and **28/*28*) and **6* (among **1/*1*, **6/*1* and **6/*6*). A significant additive effect of **6* and **28* on the decreased AUC ratio was also observed when the values for **28/*1* were compared with those for **28/*6* (Fig. 2 and Table 3).

Regarding other polymorphisms, a statistically nonsignificant tendency to decrease the AUC ratio was observed for **60*

**Table 1 Characteristics of Japanese cancer patients in this study**

| | | No. of participants | |
|---|---|---|---|
| Age | | | |
| Mean/range | 60.5/26–78 | 177 | |
| Sex | | | |
| Male/female | | 135/42 | |
| Performance status | 0/1/2 | 84/89/4 | |
| Combination therapy and tumor type (initial dose of irinotecan; mg/m$^2$) | | | |
| Irinotecan monotherapy | Lung (100) | 21 | |
| | Colon (150) | 28 | |
| | Others (100) | 7 | |
| With platinum-containing drug[a] | Lung (60) | 58[b] | 48 [60][c] |
| | Stomach (70) | 9 | 9 [80][c] |
| | Others (60) | 5 | 5 [80][c] |
| With 5-fluorouracil (including tegafur) | Colon (100 or 150) | 34 | |
| | Others (90 or 100) | 2 | |
| With mitomycin-C | Stomach (150) | 10 | |
| | Colon (150) | 1 | |
| With amrubicin | Lung (60) | 2 | |
| Previous treatment | | | |
| Surgery | Yes/no | 85/92 | |
| Chemotherapy | Yes/no | 97/80 | |
| Radiotherapy | Yes/no | 26/151 | |
| Smoking history | Yes/no | 29/148 | |

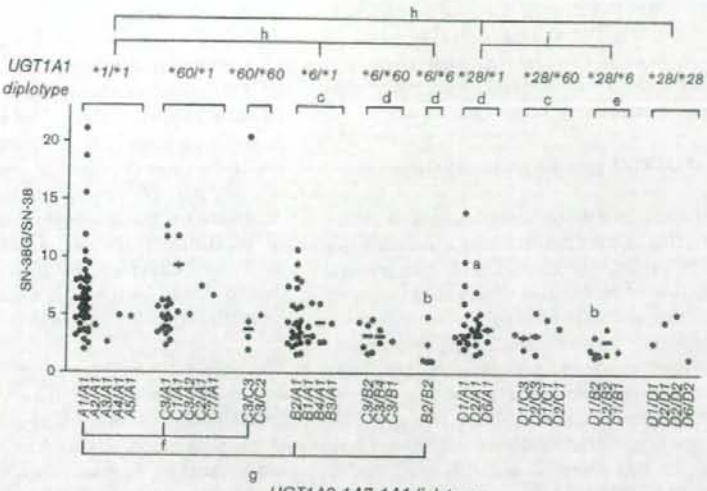
[a]Cisplatin, cisplatin plus etoposide or carboplatta.
[b]Two and eight patients received cisplatin and etoposide and carboplatin, respectively.
[c]Number of cisplatin-administered patients [initial dose of cinlatin (mg/m$^2$) is shown in brackets].

**Table 2 Combinatorial haplotypes covering *UGT1A9*, *UGT1A7*, and *UGT1A1***

| | Block haplotype[a] | | | Combination of segmental haplotypes | Cancer patients | |
|---|---|---|---|---|---|---|
| Haplotype | Block 9/6 | Block 4 | Block 3/1 | 1A9-1A7-1A1 | $N^b$ | Frequency |
| A1[c] | *I | *1 | *I | *22–*1–*1 | 189 | 0.534 |
| | *I | *3 | *I | | | |
| A3 | *III | *1 | *I | *1–*2–*1 | 2 | 0.006 |
| A2 | *II | *1 | *I | *1–*3–*1 | 1 | 0.003 |
| A4 | *IV | *1 | *I | *22–*3–*1 | 1 | 0.003 |
| A5 | | | | *T11–*1–*1 | 1 | 0.003 |
| B2[c] | *II | *1 | *III | | | |
| | *II | *1 | *VI | *1–*3–*6 | 47 | 0.133 |
| | *II | *4 | *VI | | | |
| B4 | *IV | *1 | *III | *22–*3–*6 | 6 | 0.017 |
| B1 | *I | *1 | *III | *22–*1–*6 | 5 | 0.014 |
| | *I | *1 | *VI | | | |
| B3 | *III | *1 | *III | *1–*2–*6 | 1 | 0.003 |
| C3[c] | *III | *3 | *IV | | | |
| | *III | *1 | *IV | | | |
| | *III | *3 | *V | *1–*2–*60 | 44 | 0.124 |
| | *III | *1 | *V | | | |
| C1 | *I | *3 | *IV | *22–*1–*60 | 5 | 0.014 |
| | *I | *1 | *IV | | | |
| C2 | *II | *3 | *IV | *1–*3–*60 | 2 | 0.006 |
| C7 | *VII | *3 | *V | *22–*2–*60 | 1 | 0.003 |
| D1 | *I | *1 | *IIa | *22–*1–*28 | 23 | 0.065 |
| | *I | *1 | *IIc | | | |
| D2 | *II | *1 | *IIa | | | |
| | *II | *3 | *IIa | *1–*3–*28 | 22 | 0.062 |
| | *II | *1 | *IIc | | | |
| D6 | *VI | *1 | *IIb | *1–*2–*28 | 4 | 0.011 |
| | | | | Total | 354 | 1.000 |

[a]Block haplotypes described in Ref. [28] are shown for reference. *1A9* and *1A7* are included in block 9/6 and *1A1* is included in block 3/1.
[b]Number of chromosomes.
[c]Major combinatrial haplotypes.

Fig. 2




The association of *UGT1A* diplotypes with the reduced area under concentration curve (AUC) ratio (SN-38G/SN-38) in 176 Japanese cancer patients who received irinotecan. The whole gene (*1A9-1A7-1A1*) diplotypes are shown below the abscissa and the *UGT1A1* diplotypes are indicated in the upper part of the figure. Each point represents a patient value, and the median is indicated by a bar. Significant reductions in the AUC ratio were detected in the *B2/B2*, *D2/A1*, and *D1/B2* compared with *A1/A1* for the whole gene diplotypes [Kruskal–Wallis test ($P=0.0009$) followed by Dunnett's multiple comparison test]. As for the *1A1* diplotypes, significant reductions were detected in the **6/*1*, **6/*60*, **6/*6*, **28/*1*, **28/*60*, and **28/*6* compared with the **1/*1* group [Kruskal–Wallis test ($P<0.0001$) followed by Dunnett's multiple comparison test]. Gene–dose effects on the reduced AUC ratio were significant for **6* and **28* (Jonckheere–Terpestra test). A significant additive effect of **6* on the reduced AUC ratio by **28* was detected by comparing **28/*1* and **28/*6*. $^{a}P<0.05$ and $^{b}P<0.01$ against *A1/A1* group (Dunnett's multiple comparison test); $^{c}P<0.05$, $^{d}P<0.01$, and $^{e}P<0.001$ against the **1/*1* group (Dunnett's multiple comparison test); $^{f}P<0.05$, $^{g}P<0.001$, and $^{h}P<0.0001$ (Jonckheere–Terpestra test for gene–dose effect); $^{i}P<0.01$ (Wilcoxon test).

($P=0.1134$). No significant effects on the AUC ratio were observed for Block C (exon 2–5) haplotypes or rare variations including *1A10* (**2T*, **2*, or **3*) and *1A9* (**5*, **T11*).

### Multiple regression analysis of the area under concentration curve ratio

We further assessed the impact of *UGT1A* genetic factors on the AUC ratio by multiple regression analysis. First, we used the *1A9-1A7-1A1* and Block C haplotypes as genetic factors. The AUC ratio was significantly associated with the haplotypes *B2*, *D1*, and *D2* and serum biochemistry parameters indicating hepatic or renal function before treatment. The Groups B and D haplotypes harbor *1A1*6* and **28*, respectively. The dependency on specific *1A7* or *1A9* polymorphisms, however, was not obtained, considering the contributions of both *D1* and *D2*. As *1A1*6* and **28* are mutually exclusive and their effects are comparable, we grouped *1A1*6* and **28* into the same category in the final multiple regression model (Table 4). The final model confirmed the significant contribution of this genetic marker (**6* or **28*) to the AUC ratio.

### Effects of the genetic marker '*6 or *28' on pharmaco kinetic parameters

Then, a dose effect of the genetic marker '**6* or **28*' on pharmacokinetic parameters was further analyzed

Table 3 AUC ratio of SN-38 glucuronide to SN-38 for *UGT1A1* diplotypes

| Diplotype | Number of patients | AUC ratio | | P-value[a] (vs. *1/*1) |
|---|---|---|---|---|
| | | Median | Interquartile range | |
| *1/*1 | 55 | 6.13 | 4.72–7.79 | |
| *1/*60 | 25 | 5.04 | 3.85–6.52 | 0.9803 |
| *60/*60 | 5 | 4.48 | 2.57–12.74 | 0.8141 |
| *6/*1 | 32 | 4.03 | 2.74–5.97 | 0.0126 |
| *6/*60 | 9 | 2.84 | 2.09–4.33 | 0.0021 |
| *6/*6 | 5 | 1.19 | 1.06–3.74 | 0.0012 |
| *28/*1 | 26 | 3.65 | 2.76–5.21 | 0.0040 |
| *28/*60 | 8 | 3.44 | 2.68–4.40 | 0.0261 |
| *28/*6 | 7 | 2.03 | 1.65–3.26 | <0.0001 |
| *28/*28 | 4 | 3.65 | 2.05–4.92 | 0.2322 |

AUC, area under concentration curve.
[a]Dunnett's multiple comparison test.

(Fig. 3). Patients with one haplotype harboring either **6* or **28* (**6/*1*, **6/*60*, **28/*1*, and **28/*60*) had lower SN-38G/SN-38 AUC ratios (median, 3.62; interquartile range, 2.74–5.18) than patients without **6* or **28* (**1/*1*, **60/*1*, and **60/*60*) (5.55, 4.13–7.26), and patients with two haplotypes harboring **6* or **28* (**6/*6*, **28/*28*, and **28/*6*) had the lowest AUC ratio (2.07, 1.45–3.62) ($P<0.0001$, Fig. 3a). Similarly, the number of the **6* or **28*-containing haplotypes affected the AUC ratios of SN-38 to irinotecan (Fig. 3b). When the correlations

between irinotecan dosage and the AUC of SN-38 were tested, different correlations were obtained according to the number of the haplotypes (Fig. 3c). The slope of regression line for one and two haplotypes harboring *6 or *28 was 1.4-fold and 2.4-fold greater, respectively, than that for the diplotype without *6 or *28.

### Associations of UGT1A1 genetic polymorphisms with toxicities

Association between genetic polymorphisms and toxicities was investigated in patients receiving irinotecan as a single agent. One patient was referred to another hospital 3 days after the first administration of irinotecan without evaluating toxicities and was lost in terms of follow-up. Therefore, association between genetic polymorphisms and toxicities was investigated in 55 patients. Six (11%) and 14 (25%) patients experienced grade 3 or greater diarrhea and neutropenia, respectively. As for the *1A9-1A7-1A1* diplotypes, a higher incidence of grade 3 or greater neutropenia was observed in *D1/B2* (*1A1*28/*6*) (100%, $n = 3$) than in *A1/A1* (11.8%, $n = 17$) ($P = 0.0088$, Fisher's exact test), indicating clinical impact of the genetic marker *1A1*6* or *28. As for the dose effect of '*6 or *28', incidences of grade 3 or 4 neutropenia were 14, 24, and 80% for 0, 1, and 2 haplotypes harboring these markers, respectively (Table 5). A significant association between '*6 or *28'and neutropenia was also observed for 62 patients who received irinotecan in combination with cisplatin (Table 5). No association, however, was observed between diarrhea and the marker '*6 or *28'.

### Multivariate analysis for irinotecan toxicities

We further evaluated the effect of the genetic marker '*6 or *28' on neutropenia in multivariate analysis, and confirmed a significant correlation of '*6 or *28' with the nadir of absolute neutrophil counts (Table 6). Elevated alkaline phosphatase levels and the absolute neutrophil count at baseline were also significant.

## Discussion

The association study with the *1A9-1A7-1A1* diplotypes revealed that the reduction in inactivation of SN-38, as well as neutropenia, was dependent on the Groups B and D haplotypes which corresponded to the *1A1*6* and *28 segmental haplotypes. Also, multivariate analyses clearly showed clinical significance of the genetic marker '*6 or *28' for both pharmacokinetics and toxicity of irinotecan in Japanese patients (Tables 3 and 6). *UGT1A1*6* and *28 were mutually exclusive [14] and contributed to the reduction in glucuronidation of SN-38 to the same extent. Therefore, the activity of SN-38 glucuronidation in individuals depended on the number of the haplotypes harboring *6 or *28. Although the role of *1A1*28* for irinotecan toxicity has been focused on [8–12], this study strongly suggests that *6 should be tested in addition to *28 before starting chemotherapy with irinotecan in Japanese patients.

**Table 4 Multiple regression analysis toward the AUC ratio (SN-38G/SN-38)[a]**

| Variable | Coefficient | F-value | P-value | $R^2$ | Intercept | N |
|---|---|---|---|---|---|---|
| | | | | 0.410 | 0.8869 | 176 |
| *6 or *28 | −0.189 | 70.2 | <0.0001 | | | |
| Age | 0.005 | 8.88 | 0.0033 | | | |
| Serum albumin level[b] | −0.136 | 9.92 | 0.0019 | | | |
| Serum GOT and ALP[c] | 0.070 | 8.88 | 0.0033 | | | |
| Serum creatinine[d] | 0.210 | 7.23 | 0.0079 | | | |

ALP, alkaline phosphatase; AUC, area under concentration curve.
[a]The values after logarithmic conversion were used as an objective variable.
[b]The absolute value (g/dl) before irinotecan treatment.
[c]Grade 1 or greater scores in both serum GOT and ALP before irinotecan treatment.
[d]Grade 1 or greater scores in serum creatinine before irinotecan treatment.

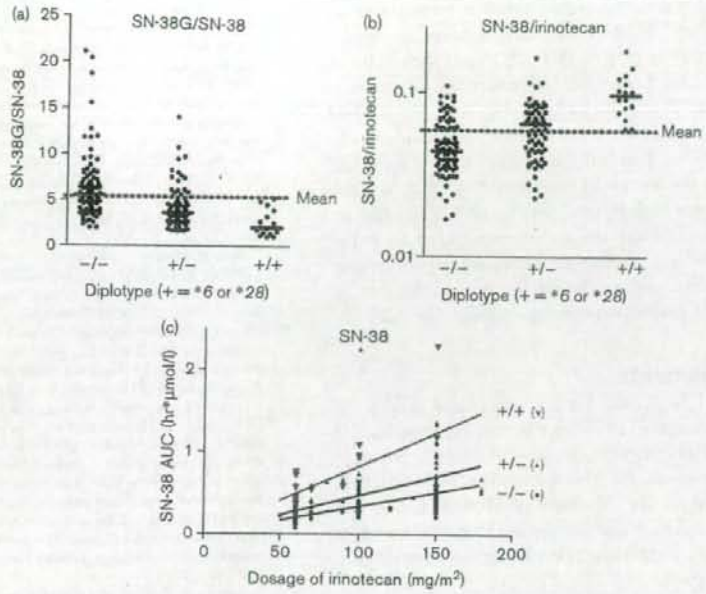
The clinical importance of *6 for neutropenia by irinotecan was also supported by a recent report in Korean patients who received irinotecan and cisplatin [31]. Although no patients with irinotecan as a single agent were homozygous for *6 in our study, clinical significance of the double heterozygote, *6/*28, was clearly demonstrated. Among patients treated with irinotecan in combination chemotherapy, the majority of patients received platinum agents in our study. A significant association of '*6 or *28' with a higher incidence of grade 3 or 4 neutropenia was also observed in patients who received irinotecan and cisplatin (Table 5). These findings further support the necessity of testing '*6 or *28' before irinotecan is given to patients.

As possible enhancement of toxicities by the *27 allele was suggested [8], we evaluated the effect of the *28c haplotype, which had an additional single-nucleotide polymorphism [*27; 686C > A(P229Q)] to the *28 allele (-40_-39insTA). In our cohort of patients, there were three *28c heterozygotes (*28c/*1) and one double heterozygote (*28b/*28c). The values of the AUC ratio were within the range of variations of the *28 group, and no additional impact of *28c was observed in relation to toxicities.

Although the decreasing trend of the AUC ratio for *1A1*60* (and combinatorial haplotype *C3*) was observed (Fig. 2), the contribution of *1A1*60* to toxicities was not clearly demonstrated in this study as reported in the Japanese retrospective study [32].

In addition to UGT1A1, recent studies have suggested possible contributions of UGT1A7, 1A9, and 1A10 to SN-38G formation [15–17]. An in-vitro study demonstrated that *1A7*3* [387T > G(N129K), 391C > A(R131K), 622T > C(W208R)] had reduced activity in terms of SN-38G formation [16]. Results of clinical studies, however, on the association between *1A7* polymorphisms and irinotecan toxicity/efficacy are inconsistent, whereas different populations with different combination therapies were used [19,20]. Furthermore, it was reported that the *UGT1A7* polymorphisms (*2 and *3), which were linked to *1A9*1*, were associated with a lowered incidence

**Fig. 3**




Effects of the genetic marker of *UGT1A1* '*6 or *28' on the area under concentration curve (AUC) ratios of SN-38G/SN-38 (a) and SN-38/irinotecan (b), and SN-38 by irinotecan dosage (c) in 176 Japanese cancer patients after irinotecan treatment.

**Table 5 Association of *UGT1A1*6* and *28 with irinotecan toxicities**

| Diplotype (+ =*6 or *28) | Number of patients | Diarrhea (grade 3) | Neutropenia (grade 3 or 4) |
|---|---|---|---|
| Irinotecan monotherapy | | | |
| −/− | 21 | 3 (14.3%)[a] | 3 (14.3%) |
| +/− | 29 | 2 (6.90%) | 7 (24.1%) |
| +/+ | 5 | 1 (20.0%) | 4 (80.0%) |
| *P*-value[b] | | 0.8500 | **0.0117** |
| *P*-value[c] | | 0.3889 | **0.0124** |
| With cisplatin | | | |
| −/− | 35 | 1 (2.9%) | 20 (57.1%) |
| +/− | 20 | 2 (10.0%) | 14 (70.0%) |
| +/+ | 7 | 1 (14.3%) | 7 (100%) |
| *P*-value[b] | | 0.1747 | **0.0315** |
| *P*-value[c] | | 0.3886 | 0.0863 |

[a]Percentage of the patient number in each diplotype is indicated in parentheses.
[b]Chi-squared test for trend.
[c]Fisher's exact test, (−/− and +/−) vs. +/+.

**Table 6 Multiple regression analysis of the nadir of absolute neutrophil counts in the patients with irinotecan monotherapy**

| Variable | Coefficient | F-value | *P*-value | $R^2$ | Intercept | *N* |
|---|---|---|---|---|---|---|
| | | | | 0.3942 | 643 | 53 |
| Serum ALP[a] | −349.9 | 12.2 | 0.0010 | | | |
| Neutrophil count before irinotecan treatment | 0.2466 | 13.5 | 0.0006 | | | |
| *6 or *28 | −369.1 | 6.40 | 0.0146 | | | |

[a]Grade 1 or greater scores of serum ALP before irinotecan treatment.

of diarrhea in the irinotecan/capecitabine regimen, in which diarrhea was a major toxicity [20]. A highly frequent allele *1A9*22* with an insertion of T into the nine T repeats in the promoter region (-126_-118$T_9 > T_{10}$) was shown to have an enhanced promoter activity in an in-vitro reporter assay [21], whereas 1A9 protein expression levels did not change in the clinical samples [22]. Rare variations, *1A9*5* [766G > A(D256N)] and *UGT1A10*3* [605C > T(T202I)], were shown to cause reduced activity *in vitro*, but their clinical importance is still unknown [23,24]. Moreover, close linkages among *1A9*, *1A7*, and *1A1* polymorphisms were found in Caucasians and Asians in an ethnic-specific manner [20,25–28].

Our study also revealed close linkages between *1A9*22* and *1A7*1*, *1A7*3* and *1A1*6* or **28* [28]. This fact makes it difficult to draw firm conclusions about the effects of *1A7*3* and *1A9*22* themselves. It is, however, reasonable to conclude that the degree of neutropenia depends on the activity of UGT1A1, because UGT1A1 is a major UGT1A enzyme in the liver and plays a primary role for regulating plasma concentrations of SN-38.

Taken together, for practical application to individualized irinotecan therapy, genotyping of *UGT1A1*6* and **28* would be beneficial and necessary in Japanese cancer patients to avoid severe adverse reactions. The frequency

of homozygotes for '**6* or **28*' (namely, **6/*6*, **6/*28*, and **28/*28*) is approximately 10%, which is comparable to the frequency of **28* homozygotes in Caucasian populations. In our study, it may be difficult to establish definite guidelines for dose reductions of irinotecan for patients homozygous for '**6* or **28*'. Considering, however, 2.4-fold steep relationship between the dose of irinotecan and the AUC of SN-38 for patients homozygous for '**6* or **28*' compared with patients without '**6* or **28*' (Fig. 3c), the dose for patients homozygous for '**6* or **28*' should be reduced to a half of the dosage recommended for other patients. Prospective studies are necessary to confirm the validity of the recommendation for dose reduction in Japanese cancer patients homozygous for '**6 or *28*'.

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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 (Arg162 Gln)] (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,

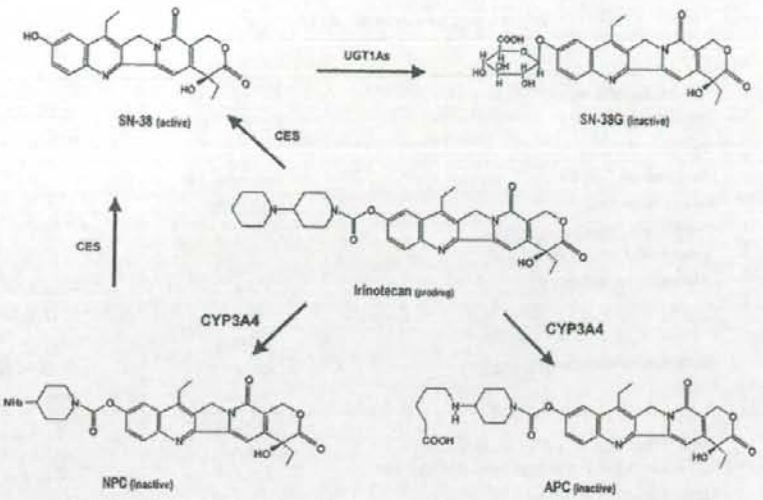



**Fig. 1** Irintoecan metabolism in human liver. CYP3A4 mediates oxidation of irinotecan to produce inactive compounds, such as APC (a major CYP3A4-mediated product) and NPC (a minor product)

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 |

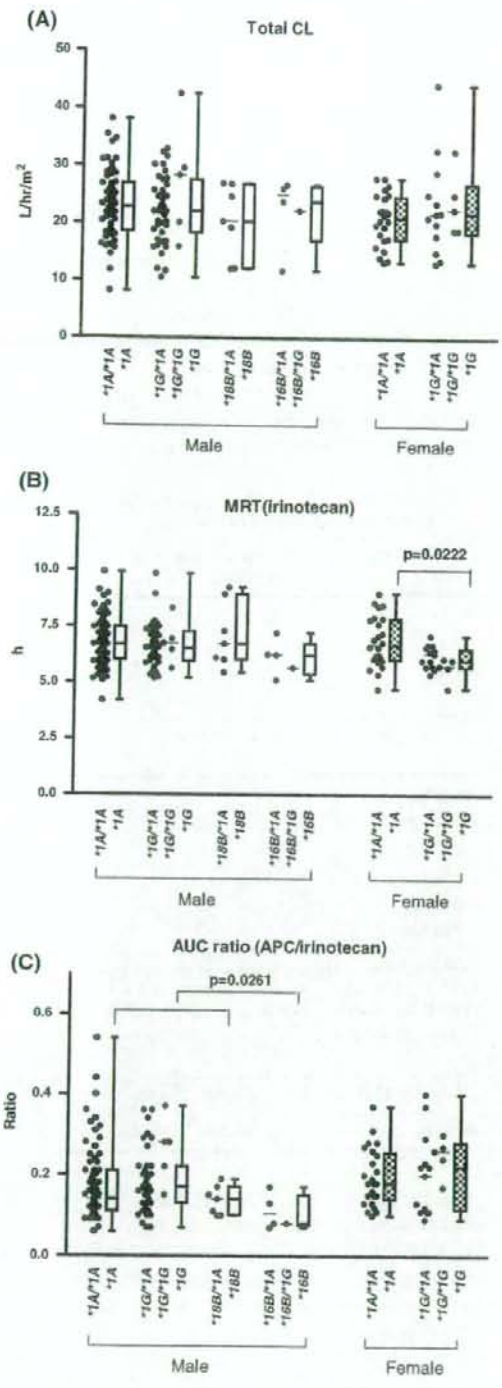
[a] Groups based on tagging SNPs of major haplotypes previously defined [4]; **1A* wild type, **1G* IVS10 + 12G > A; **16B* 554C > G (Thr185Ser) and IVS10 + 12G > A; **18B* 878T > C (Leu293Pro) and IVS10 + 12G > A

**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 $\pm$ SE $= -0.18 \pm 0.077$, $P = 0.021$) and **1G* ($0.047 \pm 0.021$, $P = 0.029$) 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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*Short Communication*

# Anti-tumor Effect of All-*Trans* Retinoic Acid Loaded Polymeric Micelles in Solid Tumor Bearing Mice

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***Purpose.*** All-*trans* retinoic acid (ATRA) polymeric micelles were developed for parenteral administration. The distribution characteristics and antitumor activities of ATRA polymeric micelles were evaluated after intravenous administration to mice bearing CT26 solid tumors.
***Methods.*** ATRA incorporated in poly(ethylene glycol)-poly(benzyl aspartate) block copolymer was prepared by the evaporation method. The levels of [$^3$H]ATRA in blood and tissue including tumor were determined by measuring the radioactivity after injection into mice. The tumor volume and the survival of the mice were determined to assess the anticancer activity.
***Results.*** The delivery of ATRA by polymeric micelles prolonged the blood circulation and enhanced the accumulation of ATRA in the tumor tissue compared with the administration of free ATRA. Tumor growth was significantly delayed and the survival time of mice was prolonged following the treatment by ATRA polymeric micelles demonstrating the improved anticancer activity of ATRA.
***Conclusion.*** Polymeric micelles are a promising and effective carrier of ATRA in order to enhance tumor delivery and they have a promising potential application in the treatment of solid tumors.

**KEY WORDS:** all-*trans* retinoic acid; antitumor activity; biodistribution; drug targeting; nanomedicine; polymeric micelles.

## INTRODUCTION

All-*trans* retinoic acid (ATRA) is an active metabolite of retinoids that has been shown to exert anti-cancer activities in a number of cancer cells and tissues. The pharmacology effects of ATRA are mainly mediated by nuclear retinoid receptor that is retinoic acid receptors, leading to growth inhibition, differentiation, and apoptosis of cancer cells (1–3). Recently, it has been extensively used for the treatment of acute promyelotic leukemia (APL) (4). However, a gradual decrease in the ATRA concentration in the blood circulation after prolonged treatment and highly variable bioavailability after oral administration were observed (5,6). Therefore, parenteral formulations that maintain the ATRA concentration in the blood could enhance its pharmacological effect.

Because of the low aqueous solubility of ATRA, drug delivery carriers such as liposomes (7), emulsions (8), and solid lipid nanoparticle (9) have been employed to improve its potency and duration of activity. In particular, clinical trials have demonstrated that liposomal ATRA offers potential pharmacological advantages over the oral administration of ATRA and appears to be an effective carrier of ATRA for APL therapy (6). Recently, we have demonstrated the inhibition of pulmonary and hepatic metastasis by ATRA incorporated in cationic liposomes (10,11) and O/W emulsions (12), respectively. However, sustained circulation of the carrier for ATRA is required for maintaining the required therapeutic level and reaching the target pathological sites in the body. Therefore, the delivery of ATRA by a micelle-forming agent is represents a novel parenteral delivery system for ATRA.

Polymeric micelles are a class of micelles that are formed from block copolymers typically consisting of hydrophilic and hydrophobic polymer chains (13). They are of particular interest because of their small particle size, their efficiency in entrapping a satisfactory amount of hydrophobic drug within the inner core, their stability in the circulation and their ability to gradually release the drug (14). Furthermore, the highly hydrated outer shells of the micelles prevent reticuloendothelial system (RES) uptake and inhibit intermicellar aggregation of their hydrophobic inner cores (15). The characteristics of these polymeric micelles could be advantage for passive delivery and extravasate the drug in the tumor site by an enhanced permeability and retention effect (EPR effect) (16). In this regard, polymeric micelles have been employed to enhance *in vivo* anticancer activity by solid tumor targeting of many anticancer drugs such as doxo-

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