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ORIGINAL ARTICLE

*UGT1A1*28* polymorphism as a determinant of irinotecan disposition and toxicity

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ABSTRACT

The metabolism of irinotecan (CPT-11) involves sequential activation to SN-38 and detoxification to the pharmacologically inactive SN-38 glucuronide (SN-38G). We have previously demonstrated the role of UGT1A1 enzyme in the glucuronidation of SN-38 and a significant correlation between *in vitro* glucuronidation of SN-38 and *UGT1A1* gene promoter polymorphism. This polymorphism (*UGT1A1*28*) is characterized by the presence of an additional TA repeat in the TATA sequence of the *UGT1A1* promoter, $((TA)_7TAA$, instead of $(TA)_6TAA$). Here we report the results from a prospective clinical pharmacogenetic study to determine the significance of *UGT1A1*28* polymorphism on irinotecan disposition and toxicity in patients with cancer. Twenty patients with solid tumors were treated with a 90 min i.v. infusion of irinotecan (300 mg m^{-2}) once every 3 weeks. The frequency of *UGT1A1* genotypes was as follows: 6/6—45%, 6/7—35% and 7/7—20%, with allele frequencies of 0.375 and 0.625 for $(TA)_7TAA$ and $(TA)_6TAA$, respectively. Patients with the $(TA)_7TAA$ polymorphism had significantly lower SN-38 glucuronidation rates than those with the normal allele (6/6>6/7>7/7, $P = 0.001$). More severe grades of diarrhea and neutropenia were observed only in patients heterozygous (grade 4 diarrhea, $n = 1$) or homozygous (grade 3 diarrhea/grade 4 neutropenia, $n = 1$ and grade 3 neutropenia, $n = 1$) for the $(TA)_7TAA$ sequence. The results suggest that screening for *UGT1A1*28* polymorphism may identify patients with lower SN-38 glucuronidation rates and greater susceptibility to irinotecan induced gastrointestinal and bone marrow toxicity.
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Keywords: irinotecan; glucuronidation; SN-38; UGT1A1; polymorphism

INTRODUCTION

The topoisomerase I inhibitor, irinotecan (CPT-11, 7-ethyl-10-[4-(1-(piperidino)-1-piperidino]carbonylcamptothecin, Camptosar®) is currently approved for use as first-line therapy in metastatic colorectal cancer, in combination with 5-fluorouracil (5-FU) and leucovorin (LV).[1] Chemotherapy with irinotecan is often accompanied by unpredictable, dose-limiting and life-threatening diarrhea and myelosuppression, attributed to its active metabolite, SN-38 (7-ethyl-10-hydroxycamptothecin). We have previously shown that SN-38 undergoes glucuronide conjugation to the pharmacologically inactive SN-38 glucuronide (SN-38G, 10-*O*-glucuronyl-SN-38) by the hepatic uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) enzyme.[2]

The glucuronidation of SN-38 may be protective against irinotecan-induced gastrointestinal toxicity.[3] Our hypothesis is that this protection may be genetically determined, as UGT1A1 enzyme is known to exist in polymorphic states. Deficient or reduced expression levels of *UGT1A1* are observed in familial hyper-

bilirubinemic syndromes such as Crigler–Najjar (Type I)[4] and Gilbert's syndrome,[5] respectively. A dinucleotide repeat polymorphism in the TATA sequence of the promoter region of *UGT1A1* has been described in Gilbert's syndrome.[5] The specific polymorphism (*UGT1A1*28*) is the presence of a $(TA)_7TAA$ sequence in the promoter region, instead of $(TA)_6TAA$. In studies with human liver microsomes, we have demonstrated an association between this polymorphism and SN-38 glucuronidation, with significantly lower SN-38 glucuronidation rates in liver samples heterozygous or homozygous for the $(TA)_7TAA$ polymorphism.[6] The current study is a prospective pharmacogenetic investigation of the influence of the *UGT1A1*28* polymorphism on the disposition and toxicity of irinotecan in patients with cancer.

RESULTS

Of the 20 patients, nine (45%) patients were of genotype 6/6, seven (35%) patients were of genotype 6/7, and four (20%) were of genotype 7/7 (Table 1). Allele frequencies of $(TA)_6TAA$ and $(TA)_7TAA$ were 0.625 and 0.375, respectively. This agrees with previous reports on the frequency of the $(TA)_7TAA$ allele of 0.33–0.39[6–8] in a Caucasian population. The other TA repeats ($(TA)_5TAA$ and $(TA)_8TAA$), shown to be present mainly in African subjects,[6,7,9] were not found in this study. The subjects in our study were predominantly of Caucasian origin (n = 18) with one patient each of African (with genotype 6/7) and Asian (with genotype 6/6) descent.

The time-course of the ratio of plasma concentration of SN-38G to SN-38 {|SN-38G|/|SN-38|} (Figure 1) indicated much lower glucuronidation rates of SN-38 for patients with genotypes 6/7 and 7/7 when compared to those that are homozygous for the wild-type allele (6/6) at each blood sampling time. There was a significant progressive reduction in SN-38 glucuronidation rates in patients who are heterozygous (6/7) and homozygous (7/7) for the $(TA)_7TAA$ polymorphism (Table 1) (6/6>6/7>7/7, z = –2.93, P = 0.001). Circulating levels of SN-38 were higher in patients with the $(TA)_7TAA$ polymorphism, reflected by the significant increase in $AUC_{SN\text{-}38}$ values in patients with 6/7 and 7/7 genotype (7/7>6/7>6/6, z = 3.23, P = 0.001, Table 1). Patients with 6/7 and 7/7 genotypes had lower $AUC_{SN\text{-}38G}$ values than those with the 6/6 genotype, although this decrease was not statistically significant (Table 1). As previous studies on the weekly schedule of irinotecan have indicated a significant relationship of biliary index of SN-38 with severity of diarrhea,[3] we examined the association between biliary index and *UGT1A1*28* polymorphism. Patients with genotypes 6/7 and 7/7 had a significant trend toward higher biliary indices (7/7>6/7>6/6, z = 3.23, P = 0.001).

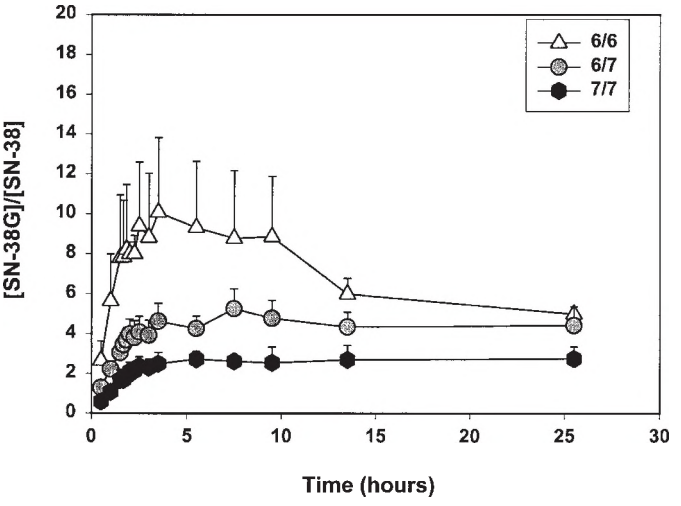

Figure 1 Time-course of plasma concentration ratios of SN-38G to SN-38 {[SN-38G]/[SN-38]} in patients after irinotecan treatment, stratified according to *UGT1A1* promoter genotype. All data are the mean ratio in respective number of patients in each group—6/6 (n = 9), 6/7 (n = 7) and 7/7 (n = 4) ± SEM.

With regard to toxicity, ANC nadirs significantly correlated with AUC values for SN-38 (r = −0.81, P < 0.0001). There was a significant trend toward lower ANC nadir values in patients with genotypes 6/7 or 7/7 (Figure 2). All patients with two wild-type alleles (6/6) had no/low diarrhea and neutropenia (grades 0 or 1). More severe grades of diarrhea and neutropenia were observed only in patients with genotypes 6/7 (one patient with grade 4 diarrhea) and 7/7 (one patient with grade 3 diarrhea and grade 4 neutropenia and

Table 1 Frequency of *UGT1A1* promoter genotypes and comparison of dispositional parameters of SN-38, the active metabolite of irinotecan

UGT1A1 genotype	*Number*	*Frequency*	*SN-38 glucuronidation ratio*[a]	$AUC_{0\text{-}t}$ *(SN-38)*[b] *(ng h^{-1} ml^{-1})*	$AUC_{0\text{-}t}$ *(SN-38G) (ng h^{-1} ml^{-1})*	*Billiary index*[c]
6/6	9	0.45	9.28 (3.77)	205.13 (23.52)	1631.54 (459.67)	2728.82 (452.36)
6/7	7	0.35	4.04 (0.65)	288.61 (25.51)	1144.44 (200.59)	4970.91 (1242.31)
7/7	4	0.20	2.41 (0.54)	531.37 (169.82)	1236.96 (397.70)	9685.61 (2411.57)

All data are expressed as mean (SEM).
[a]6/6>6/7>7/7, z = –2.93, P = 0.001; [b]7/7>6/7>6/6, z = 3.23, P = 0.001; [c]7/7>6/7>6/6, z = 3.23, P = 0.001, non-parametric trend analysis.

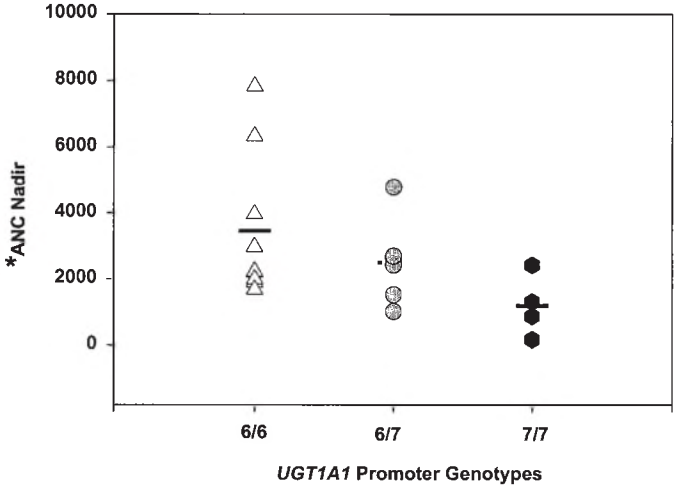

Figure 2 Correlation between absolute neutrophil count (ANC) and *UGT1A1* promoter genotypes in patients after irinotecan treatment. *Significant trend in ANC nadirs (6/6>6/6>7/7, $z = -2.05$, $P = 0.04$, non-parametric trend analysis).

one patient with grade 3 neutropenia). However, this difference was not significant among the three genotypes ($P = 0.242$ for neutropenia grades and $P = 0.289$ for diarrhea grades).

DISCUSSION

The major dose-limiting toxicities of irinotecan include diarrhea and myelosuppression (to a lesser extent). Incidences of irinotecan-induced gastrointestinal toxicity can be severe and do not respond adequately with conventional anti-diarrheal treatments.[10] Irinotecan therapy is also complicated by the significant inter-subject variations in such toxicity and pharmacokinetic parameter estimates.[3,11] As glucuronidation of the active metabolite, SN-38, is a major determinant of irinotecan metabolism and toxicity,[2,3] we have investigated the contribution of genetic differences in UGT1A1 enzyme activity on irinotecan disposition and toxicity. Previous studies have indicated severe CPT-11 toxicity in patients with Gilbert's syndrome, diagnosed by increased serum bilirubin levels.[12] In a study with nine patients, Ando *et al*[13] observed a lower glucuronidation rate of SN-38 in a patient with 7/7 genotype *vs* those with 6/7 ($n = 1$) and 6/6 ($n = 7$) genotypes. A retrospective study in Japan indicated that the 6/7 and 7/7 genotypes would be a significant risk factor for severe irinotecan toxicity.[14] The current study is the first prospective clinical investigation addressing the influence of pharmacogenetics of *UGT1A1* polymorphism on therapy with irinotecan.

Our results indicate that screening of patients for the *UGT1A1*28* polymorphism may be a useful diagnostic tool to predict *in vivo* glucuronidation of SN-38. Patients with the $(TA)_7TAA$ repeat in the promoter region of *UGT1A1* may be expected to exhibit a higher incidence of diarrhea and neutropenia than those without the polymorphism. A recent report has indicated an unexpectedly high rate of early deaths due to dehydration, neutropenia and sepsis after initiation of treatment with irinotecan combined with 5-FU/LV.[15] The results from our study may at least in part explain the reasons for such toxicity, which may be genetically determined.

We have recently extended this study to increase the irinotecan dose to 350 mg m^{-2} with a sample size of 60 patients. Besides the dinucleotide repeat polymorphism in the promoter region, single nucleotide polymorphisms (SNPs) in the coding region of *UGT1A1* are also being investigated, such as 211G→A, 686C→A, 1091C→T, which have been shown to be present only in Asian subjects.[16,17] The Asian subject in our study who had a genotype of 6/6 (and SN-38 glucuronidation ratio of 6.15) did not have the 211G→A (Gly71Arg) mutation that is present at high frequency (32%) in Asian subjects.[18] Other possible pharmacogenetic determinants of irinotecan toxicity such as polymorphisms in metabolizing enzymes (CYP3A4/3A5 and carboxylesterase)[19] and transporters (p-glycoprotein)[20,21] are also being investigated in this study. Future studies should also address the relationship between the *UGT1A1* genotype and response in colorectal cancer and other irinotecan-sensitive neoplasms.

METHODS

Patients

Twenty adult patients (10 male, 10 female) with solid tumors (lung—seven, colon—three, esophageal and liver—two each, and one each of chondrosarcoma, nasopharyngeal, endometrial, ovarian, rectal and neuroendocrine tumors) participated in this study. Standard phase I clinical eligibility criteria were used to enroll patients including adequate performance status (≥70% Karnofsky score), recovery from toxicity from previous therapy and measurable or evaluable disease. Inclusion criteria included WBC ≥3500, ANC ≥1500, platelets ≥100000 μl^{-1}, creatinine ≤1.5 mg dl^{-1}, normal conjugated bilirubin levels, and SGOT, SGPT $<5 \times$ upper limit of normal unless due to disease. Patients with biologic or chemotherapy within 4 weeks (6 weeks for prior treatment with nitrosoureas or mitomycin C) prior to irinotecan treatment were excluded from the study. Patients with prior radiation therapy within 4 weeks (if greater than 25% of bone marrow was irradiated) or on colony stimulating factors within 2 weeks were excluded. Post-transplant patients, women of childbearing potential not practising birth control, pregnant or lactating women were also excluded. In addition, patients with history of inflammatory bowel disease requiring therapy, chronic diarrheal episodes or paralytic ileus were excluded from the study.

To prevent nausea and vomiting, all patients were pretreated with ondansetron (Zofran®) 20 mg, IVPB (in 50 cc of 5% dextrose injection, USP, given over 0.5 h), 30 min prior to irinotecan administration. All patients were cautioned about possible severe diarrhea and were given written instructions to begin loperamide (Imodium®) treatment for late diarrhea (occurring more than 24 h after irinotecan administration) at a dose of 4 mg at the first onset of late diarrhea and then 2 mg every 2 h (or every 4 h while asleep) until the patient is diarrhea-free for at least 12 h. Irinotecan

dose was reduced by 50 mg m^{-2} in patients experiencing grade 3 or 4 toxicities, including diarrhea and neutropenia.

Informed consent was obtained from each patient for all study procedures including the collection of DNA and genetic analysis, in accordance with Federal and institutional guidelines.

Irinotecan Formulation and Dosing

Irinotecan solution (20 mg ml^{-1}) was supplied by the National Cancer Institute. Appropriate dilutions were made in 5% dextrose solution and administered to patients by intravenous infusion (90 min) at a dose of 300 mg m^{-2}, once every 3 weeks. This dose was selected prior to acceptance of 350 mg m^{-2} as the preferred dose on the once every 3-week schedule.[22]

Blood Sampling

Blood samples (7 ml) were collected on day 1 of cycle 1 at baseline and various time intervals—30, 60 and 90 min during infusion and at 10, 20, 30, 45, 60 min and 1.5, 2, 4, 6, 8, 12 and 24 h after infusion of irinotecan. Plasma samples were separated and analyzed for the determination of concentrations of irinotecan, SN-38 and SN-38G. A blood sample (4.5 ml) was also collected at baseline for the isolation of DNA and *UGT1A1* genotyping.

Analysis of Irinotecan, SN-38 and SN-38G in Plasma

Plasma concentrations of irinotecan and its metabolites (SN-38 and SN-38G) were measured using a reverse phase HPLC method with fluorescence detection.[11] Briefly, plasma samples (100 μl) were centrifuged (2500 *g* for 20 min) after addition of methanol and internal standard (camptothecin, 1 μg ml^{-1}). The supernatant was evaporated to dryness and reconstituted in 200 μl mobile phase, consisting of 70% 0.1 M potassium dihydrogen phosphate with 3 mM 1-heptanesulfonic acid (pH 4.0) and 30% acetonitrile. One hundred microliter-aliquots were injected into an HPLC system (Hitachi Instruments, San Jose, CA, USA) coupled to a fluorescence detector (λ_{ex} = 375 nm and λ_{em} = 566 nm) and a μBondapak C_{18} column (10 μm, 3.9 × 300 mm; Waters Corporation, MA, USA) with a mobile phase flow rate of 0.8 ml min^{-1}. SN-38G concentrations were determined indirectly as the increase in SN-38 concentrations after hydrolysis (2 h, 25°C) of SN-38G with β-glucuronidase enzyme (*E. coli* Type IX-A, Sigma-Aldrich Co, MO, USA) (1000 U ml^{-1} in 0.1 M sodium phosphate buffer, pH 6.4).

UGT1A1 Genotyping Assay

UGT1A1 genotyping was performed after amplification of isolated DNA samples by polymerase chain reaction with primers flanking the polymorphic TA locus (forward primer: 5′-GTCACGTGACACAGTCAAAC-3′ and reverse primer: 5′-TTTGCTCCTGCCAGAGGTT-3′), described previously.[6,8] Genotypes were assigned as follows: 6/6—homozygous for $(TA)_6$TAA: 6/7—heterozygous with each of $(TA)_6$TAA and $(TA)_7$TAA; and 7/7—homozygous for $(TA)_7$TAA.

Toxicity Measurement

Toxicity was measured using nadir values of absolute neutrophil counts (ANC) after the first irinotecan dose (day 12–14) and grades of diarrhea and neutropenia, which were on a scale of 0–4, as per NCI guidelines on grading common toxicities after chemotherapy (CTC version 2.0, http://ctep.info.nih.gov).

Data Analysis

Pharmacokinetic parameters were determined using non-compartmental analysis (WinNonlin®, Pharsight Corp, Apex, NC, USA). The *in vivo* glucuronidation rate of SN-38 (SN-38 glucuronidation ratio) was expressed as the ratio of the area under the plasma concentration-time curve until the last sampling point (AUC) for SN-38G ($AUC_{SN\text{-}38}G$) over that for SN-38 ($AUC_{SN\text{-}38}$). Biliary index of SN-38, which is a surrogate measure of SN-38 biliary excretion was calculated as $(AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}) \times AUC_{CPT\text{-}11}$, as described previously.[3]

Statistical Analysis

Statistical analysis was performed using the Stata® software package (Stata Corp, College Station, TX, USA). Nonparametric trend analysis[23] was conducted to compare pharmacokinetic parameters, glucuronidation ratios, biliary indices and ANC nadirs among patients with the three *UGT1A1* genotypes. Fisher's exact test was used for testing of statistical significance in diarrhea and neutropenia grades between genotypes. *P*-values ≤0.05 were regarded as statistically significant and all tests were two-sided. The sample size of 20 represents the cohort of patients treated at the dose of 300 mg m^{-2}; the protocol was subsequently amended to utilize the currently accepted dose of 350 mg m^{-2} and evaluation at that dose level is ongoing.

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NOTES

All data will be deposited in The Pharmacogenetics Knowledge Base (PharmGKB, U01GM61374, http://pharmgkb.org/).

DUALITY OF INTEREST

The authors would like to disclose that there are patents pending in addition to the following: 'Methods for detection of promoter polymorphism in a UGT gene promoter', Mark J Ratain, MD, Lalitha Iyer, PhD and Anna DiRienzo, PhD (patent pending).

ABBREVIATIONS

6/6	*homozygous for $(TA)_6$TAA*
6/7	*heterozygous with one each of alleles $(TA)_6$TAA and $(TA)_7$TAA*
7/7	*homozygous for $(TA)_7$TAA*
ANC	*acute neutrophil count*
AUC	*area under the plasma concentration-time curve*
CPT-11	*irinotecan (CPT-11,7-ethyl-10-[4-(1-(piperidino)-1-piperidino] carbonylcamptothecin)*
SN-38	*7-ethyl-10-hydroxycamptothecin*
SN-38G	*SN-38 glucuronide (10-O-glucuronyl-SN-38)*
UGT1A1	*uridine diphosphate glucuronosyltransferase, isoform 1A1.*

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