Significant Correlation between Polymorphisms of *UGT1A1* Gene and Low Irinotecan Toxicity in Colorectal Cancer Patients with FOLFIRI

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Abstract: *Aim:* To investigate the association between UDP-glucuronosyltransferase 1A1 (*UGT1A1*) genotypes and severe toxicity in Taiwanese patients with metastatic colorectal cancer (mCRC) receiving irinotecan chemotherapy.

Methods: We genotyped the *UGT1A1* gene by direct sequencing. All the patients were evaluated to see whether the variant *UGT1A1* genotype would correlate to severe toxicity of irinotecan consisting of grade III-IV neutropenia, diarrhea and nausea/vomiting. Genomic DNA was genotyped for *UGT1A1*, and patients were designated as 6/6, 6/7, or 7/7 depending on the number of TA repeats in the promoter region.

Results: The results showed that the genotype distribution of *UGT1A1* in Taiwanese subjects differed significantly from that in Caucasians. Furthermore, patients with 6/7 or 7/7 genotype were associated with a higher incidence of grade III-IV neutropenia or diarrhea or nausea/vomiting (all $p < 0.0001$). The less frequencies of 6/7 and 7/7 genotypes may be responsible for the considerably lower occurrence of grade III-IV neutropenia and diarrhea in Taiwanese patients. Indeed, the *UGT1A1* genotype was closely related to clinical response ($p = 0.018$).

Conclusion: UGT1A1 genotyping is a potential predictor of severe toxicity for Taiwanese mCRC patients treated with irinotecan chemotherapy, and may be useful to identify patients at-risk of toxicity, and thus could be used as a screening tool prior to therapy.

Key Words: UGT1A1, irinotecan, toxicity, metastatic colorectal cancer, FOLFIRI.

INTRODUCTION

Variation in the genetic constitution between individuals will have a major impact on the activation and metabolism of certain chemotherapeutic agents. Single-nucleotide polymorphisms (SNPs) account for over 90% of genetic variation in the human genome. Irinotecan, a semisynthetic camptothecin analog with topoisomerase I–inhibiting activity [1-3], shows excellent clinical efficacy in solid tumors such as lung, ovarian, and colorectal cancers (CRC) [4-6]. Irinotecan is a prodrug that is converted by carboxylesterase to an active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38), which is 100- to 1,000-fold more cytotoxic than irinotecan. SN-38 can be inactivated through glucuronidation by a member of the uridine diphosphate glucuronosyltransferase (UGTs) family in the liver to an inactive metabolite, SN-38 glucuronide (SN-38G) [2, 3]. Therefore, the conversion of SN-38 to SN-38G by hepatic UGTs is a critical step in the sequential metabolic pathway of irinotecan. Because glucuronidation is the major route of detoxification and elimination of active metabolite SN-38, inherited differences in irinotecan glucuronidation capacity may have an important influence on the pharmacokinetics and toxicity of this drug [7, 8].

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A dinucleotide repeat in a TATA box in the *UGT1A1* promoter results in altered UGT1A1 activity [9]. Reduced UGT1A1 is linked to a high risk (approximately four-fold) of severe toxicity from irinotecan treatment, including dose-limiting diarrhea and neutropenia [10]. The variable number of TA repeats ranges from five to eight copies, six TA repeats (6TA/6TA) represent the most common allele, with up to 33% in Caucasians having a variant allele containing seven repeats (*UGT1A1*28*) [11]. The clinical significance between patients with the *UGT1A1*28* allele and reduced UGT1A1 expression, and consequently reduced SN38 glucuronidation and irinotecan-related toxicity is well established [11-14]. Assessment of the presence of the *UGT1A1*28* allele in cancer patients prior to administration of irinotecan may predict individuals at risk for severe toxicity from irinotecan, allowing the selection of lower doses or alternative therapies.

Several recent studies have suggested that a statistically significant relationship is found between the appearance of severe diarrhea or/and hematological toxicities and the homozygote *UGT1A1*28* genotype when compared with *UGT1A1* wild-type Caucasian CRC patients [15-18]; however, no information in Taiwanese patients with metastatic CRC (mCRC) treated with irinotecan-based chemotherapy has ever been reported. The aim of our study was to assess the role of *UGT1A1* gene polymorphisms on the occurrence of severe toxicities and response in Taiwanese patients with mCRC receiving FOLFIRI regimen. Furthermore, we have also compared the *UGT1A1* gene polymorphisms and incidence of toxicities of mCRC in Asian and Caucasian patients receiving FOLFIRI regimen.

MATERIALS AND METHODOLOGY

Patients and Treatment

This prospective pilot study was conducted from January 2005 to March 2008, including 72 cytologically or histologically confirmed mCRC (International Union Against Cancer (UICC) stage IV) patients, and performance status (PS) $\leqq 2$ on the Eastern Cooperative Oncology Group (ECOG) scale, in the Kaohsiung Medical University Hospital receiving FOLFIRI regimen. Written informed consent was obtained from all subjects and/or guardians for the use of their blood samples. Sample acquisition and subsequent use were also approved by the institutional review board of the Kaohsiung Medical University Hospital. The FOLFIRI regimen is as follows: irinotecan (180 mg/m^2) on day 1 with Leucovorin (LV) (200 mg/m^2) administered as a 2-hour infusion before 5-fluorouracil (5-FU) (400 mg/m^2) administered as an intravenous bolus injection, and 5-FU (2400 mg/m^2) as a 46-hour infusion immediately after 5-FU bolus injection on days 1 and 2. Courses were repeated every two weeks in the presence of an absolute neutrophil count $\geq$1500/μl and platelet count $\geq$100,000/μl, and recovery of any extra-hematological toxicity; otherwise, treatment was postponed for one or two weeks until recovery. Also, the chemotherapy was continued until the disease progressed or unacceptable toxicities developed or the patient refused further treatment with FOLFIRI. Any grade III or IV adverse events resulted in an approximately 20% dose reduction of irinotecan for subsequent cycles. Persistent grade II or worse adverse events delayed therapy until recovery. The use of colony-stimulating factors was allowed if medically justified. Intensive treatment with loperamide, if needed, was used for diarrhea. All patients were included in safety and efficacy analyses. Safety assessment and laboratory tests were performed biweekly. The severity of adverse effects was evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC), version 2.0 (http://ctep.cancer.gov/reporting/ctc.html; accessed in September 2008). Baseline measurement of tumor size was based on computed tomography (CT) scan or X-ray or other radiological means. Tumors were measured at 6- to 8-week intervals, and objective response was evaluated according to the response evaluation criteria for solid tumors (RECIST) [19], as well as the best response being recorded. For the analysis, patients with a complete or partial response were grouped in responders; patients with a stable disease or progressive disease were grouped in non-responders.

Genotyping

Constitutional gene polymorphisms were analyzed by DNA extraction from 4 mL peripheral blood using PUREGENE® DNA Isolation Kit (Gentra Systems Inc., Minneapolis, MN, USA). All genomic DNA from the patients were analyzed using direct sequencing technique for the determination of genotypes of *UGT1A1* promoter region. The primers used in this study were designed by using primer 3 free software (http://web.umassmed.edu/bioapps/primer3_www.cgi; accessed in May 2008). The sequences of the forward and reverse primers were 5'- AGTCACGTGACACAGTCAAACA-3' and CTTTGCTCCTGCCAGAGGTT-3', respectively. The PCR reaction volume was 40 μl and the PCR conditions for these polymorphisms were described as follows: *GSTP1*: 94°C for 5 min; 30 cycles of denaturation for 30 sec at 94°C, annealing for 20 sec at 67.5°C, primer extension for 20 sec at 72°C, and a final extension for 10 min at 72°C. Genotype verifications were carried out by fragment analysis of the PCR product using the automated capillary electrophoresis on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, Calif., USA) and analyzed with GeneScan and Genotyper software (Applied Biosystems).

Statistical Analysis

All data were analyzed using Statistical Package for the Social Sciences Version 12.0 software (SPSS Inc., Chicago, Ill, USA). All genotypes were tested whether they were distributed according to the Hardy-Weinberg equilibrium or not. The Hardy-Weinberg equilibrium assumption was assessed by the standard method of matching the observed numbers of individuals in the different genotype categories with those expected under Hardy-Weinberg equilibrium for the estimated allele frequency. χ^2 test for deviation from Hardy-Weinberg equilibrium was used to estimate differences in allele frequencies. Differences between categorical variables were measured by the two-sided Pearson χ^2 test and Fisher's exact test. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Characteristics of Patients

Of these 72 mCRC patients, 43 were males and 29 were females. The median age was 60 years (range, 20–80). Forty-five tumors were located in the colon, and 27 tumors were in

the rectum. Two (2.8 %), 19 (26.4 %), 24 (33.3 %), and 27 (37.5 %) patients were subsequently categorized into complete response, partial response, stable disease, and progressive disease, respectively, according to RECIST. Consequently, 21 (29.2%) and 51 (70.8 %) patients were finally classified into responders and non-responders. Patients' characteristics are depicted in Table **1**.

Table 1. Baseline Characteristics of 72 Metastatic Colorectal Cancer Patients Undergoing Irinotecan-Based Chemotherapy

Variables	No. (*n* =72)
Gender	
Male	43 (59.7)
Female	29 (40.3)
Median age (range, year)	60 (24-80)
Performance status (ECOG)	
0	41 (56.9)
1	26 (36.1)
2	5 (7)
Tumor location	
Colon	45 (62.5)
Rectum	27 (37.5)
As nth-line chemotherapy	
1	34 (47.2)
2	34 (47.2)
3	4 (5.6)
Sites of metastases	
Liver	42 (58.3)
Lung	21 (29.2)
Local recurrence	17 (23.6)
Peritoneum	12 (16.7)
Bone	7 (9.7)
Distant lymph nodes	5 (7)
Others	8 (11.1)
Multiple	28 (38.9)
Objective response	
Complete response	2 (2.8)
Partial response	19 (26.4)
Stable disease	24 (33.3)
Progressive disease	27 (37.5)

Values in parenthesis are percentages. ECOG, Eastern Cooperative Oncology Group.

UGT1A1 Genotype with Toxicity and Clinical Response Among Various Races

The frequencies of 6TA/6TA, 6TA/7TA, and 7TA/7TA genotypes were 53 (73.6 %), 18 (25 %), and 1 (1.4 %) of 72 patients respectively. There was a marked relationship between the appearance of severe (Grade III/IV) neutropenia, diarrhea, and nausea/vomiting and the heterozygous (6TA/7TA) or homozygous *UGT1A1*28* (7TA/7TA) conditions (all $p < 0.0001$, Table **2**). Of these patients with severe toxicities, 87.5 % to 100 % were heterozygous and homozygous *UGT1A1*28* conditions; while in patients without severe toxicities only 18.7 % to 20.1 % were heterozygous *UGT1A1*28* genotype. Table **3** summarized the genotype and allele frequencies of *UGT1A1* among our study and other previous studies [15, 17, 20, 21]. A significant lower frequency of 6TA/7TA and 7TA/7TA genotypes in Taiwanese patients when compared to that of Caucasian patients was observed ($p < 0.0001$). However, no statistical difference of the frequency of 6TA/7TA and 7TA/7TA between our study and other Asian or Japanese studies was found. Less than one-fifth of Asian patients were 7TA allele, of which the frequency of allele distribution was significantly lower than Caucasian patients ($p < 0.0001$). A considerably higher incidence of severe neutropenia ($p = 0.011$) and diarrhea ($p < 0.0001$) in Caucasian patients than in Taiwanese patients was noted, though a significant difference was not achieved in severe nausea/vomiting ($p = 0.224$; Table **4**). Furthermore, a prominent correlation between the *UGT1A1* genotypes and clinical response to FOLFIRI chemotherapy was shown ($p = 0.018$, Table **5**). Ten of 21 (47.6 %) responders were 6TA/7TA or 7TA/TA genotypes, whereas 9 of 51 (17.6 %) non-responders were 6TA/7TA or 7TA/TA genotypes.

DISCUSSION

Due to its efficacy, irinotecan is currently approved as first-line therapy in mCRC treatment, and can cause severe unpredictable gastrointestinal and hematologic toxicity. Much of the inter-individual variability in drug toxicities is attributable to the presence of SNPs in genes encoding drug-metabolizing enzymes and drug transporters involved in the biochemical pathway of irinotecan. Although more than 50 genetic lesions in the *UGT1A1* gene have been described [22], the *UGT1A1*28* allele (the most frequent polymorphism in Caucasian populations) plays a vital role in the development of toxicity after irinotecan chemotherapy. To elucidate the most important functional polymorphism that determines the severe toxicities of Taiwanese patients with mCRC treated with irinotecan-based chemotherapy, we examined the polymorphisms of the *UGT1A1* genes involved in irinotecan-metabolizing enzymes. The present investigation shows that there is a significant difference in the genotype and allele distribution between Taiwanese and Caucasian mCRC patients. Likewise, several recently published studies have shown the disposition of irinotecan and its metabolites to differ among different ethnic groups [14,18,23,24]. When compared with other ethnic groups, the distribution of the wild-type 6TA/6TA genotype or 6TA allele in Taiwanese mCRC patients was relatively high (approximately two-fold) and similar to the Japanese and other Asian populations, but different from Caucasian patients. Notably, the 6TA allele and 7TA allele frequencies in Caucasian and Asian (including Taiwan) populations are different.

The development of severe toxicities including neutropenia, diarrhea, and nausea/vomiting is closely associated with the 6TA/7TA and 7TA/7TA genotypes in our patients treated with FOLFIRI regimen. Several recent studies have

Table 2. Association Between *UGT1A1* Polymorphism and the Occurrence of NCI-CTI Grade III and IV Toxicities

Genotype	No. (*n* = 72)	Grade III/IV Neutropenia		Grade III/IV Diarrhea		Grade III/IV Nausea/Vomiting	
		Experienced No. (*n* = 6)	Not Experienced (*n* = 66)	Experienced No. (*n* = 4)	Not Experienced No. (*n* = 68)	Experienced No. (*n* = 8)	Not Experienced No. (*n* = 64)
6/6	53 (73.6)	0 (0)	53 (80.3)	0 (0)	53 (77.9)	1 (12.5)	52 (81.3)
6/7	18 (25)	5 (83.3)	13 (19.7)	3 (75)	15 (20.1)	6 (75)	12 (18.7)
7/7	1 (1.4)	1 (16.7)	0 (0)	1 (25)	0 (0)	1 (12.5)	0 (0)
P		<0.0001		<0.0001		<0.0001	

Values in parentheses are percentages. NCI-CTI, National Cancer Institute-Common Toxicity Criteria; UGT1A1, uridine diphosphate glucuronosyl transferase.

Table 3. Comparison of Distribution of *UGT1A1*genotypes Between our Taiwanese Colorectal Cancer Patients and other Populations[*]

	Caucasian No. (*n* = 95)	Caucasian No. (*n* = 72)	Japanese No. (*n* = 118)	Asian No. (*n* = 45)	Taiwanese No. (*n* = 72)
Genotype					
6/6	40 (42.1)	31 (41)	93 (78.8)	30 (66.7)	53 (73.6)
6/7	45 (47.4)	35 (47)	18 (15.3)	15 (33.3)	18 (25)
7/7	10 (10.5)	7 (9)	7 (5.9)	0 (0)	1 (1.4)
P	< 0.0001	< 0.0001	0.101	0.294	-
Allele frequency					
6TA	0.658	0.664	0.864	0.833	0.861
7TA	0.342	0.336	0.136	0.167	0.139
P	< 0.0001	0.001	0.928	0.562	-
Authors	Marcuello [15]	Rouits [17]	Ando [20]	Jada [21]	-

Values in parentheses are percentages. *UGT1A1*, uridine diphosphate glucuronosyl transferase. [*]Authors in this table are presented with reference number ([n]) and the abbreviated name of the first author in the previously reported paper.

Table 4. Comparison of Association Between *UGT1A1* Polymorphism and the Occurrence of NCI-CTI Grade III and IV Toxicities Between our Taiwanese Colorectal Cancer Patients and Caucasians (Marcuello [15])[*]

Genotype	Grade III-IV Neutropenia		Grade III-IV Diarrhea		Grade III-IV Nausea/Vomiting	
	Caucasian No.	Taiwanese No.	Caucasian No.	Taiwanese No.	Caucasian No.	Taiwanese No.
6/6	6 (15)[†]	0 (0)[†]	7 (17)[†]	0 (0)[†]	5 (12)[†]	1 (1.9)[†]
6/7	12 (27)[†]	5 (27.8)[†]	15 (33)[†]	3 (16.7)[†]	10 (22)[†]	6 (33.3)[†]
7/7	4 (40)[†]	1 (100)[†]	7 (70)[†]	1 (100)[†]	2 (20)[†]	1 (100)[†]
Overall	22 (23.2)[†]	6 (8.3)[†]	29 (30.5)[†]	4 (5.6)[†]	17 (17.9)[†]	8 (11.1)[†]
P[‡]	0.011	-	< 0.0001	-	0.224	-

Values in parentheses are percentages of all genotyping patients. NCI-CTI, National Cancer Institute-Common Toxicity Criteria. [*]Authors in this table are presented with reference number ([n]) and the abbreviated name of the first author in the previously reported paper. [†]Indicates the percentage of all patients with the same genotype. [‡]Comparison between the overall incidences of grade III and IV toxicities.

Table 5. Correlation Between *UGT1A1* Polymorphism and Response to Chemotherapy

	Responder* (*n* = 21)	Nonresponder† (*n* = 51)	*P*
Genotype			
6/6	11 (52.4)	42 (82.4)	0.018
6/7	9 (42.9)	9 (17.6)	-
7/7	1 (4.7)	0 (0)	-

Values in parentheses are percentages. *UGT1A1*, uridine diphosphate glucuronosyl transferase, *responder, complete response and partial response; †nonresponder, stable disease and progressive disease.

evaluated the impact of the *UGT1A1* polymorphism on the main dose-limiting toxicities of irinotecan (i.e., diarrhea and neutropenia). For example, consistent with our observation, Côté and his colleagues showed that CRC patients with *UGT1A1*28* polymorphism had more frequent severe hematologic toxicity (50%) than patients homozygous for wild-type allele (12.5%) [16]. Meanwhile, Toffoli *et al.* indicated that *UGT1A1*28* polymorphism was associated with a higher risk of grade III to IV hematologic toxicity (odds ratio, 8.63; 95% CI, 1.31 to 56.55), which was only relevant for the first cycle of irinotecan chemotherapy.[18] Regarding the diarrhea, de Jong *et al*. also found that the presence of at least one *UGT1A1*28* allele was strongly related to the incidence of grade II-III diarrhea [25]. Ando *et al.* stressed that the 7TA/7TA and 6TA/7TA genotypes would be a significant risk factor for severe irinotecan toxicity [20]. Moreover, Araki *et al*. demonstrated that Japanese cancer patients who were heterozygous and homozygous *UGT1A1*28* genotypes had markedly lower SN-38 glucuronidation activity than those who were patients homozygous for wild-type, potentially increasing susceptibility to toxicity of irinotecan [26]. However, the association between *UGT1A1* polymorphism and development of adverse event is not always identical in all studies. In a recent prospective study in which irinotecan was the sole chemotherapeutic agent, a significant association was found between the *UGT1A1* genotype and neutropenia but not with diarrhea [27]; whereas in another retrospective study in which a combined irinotecan regimen was used, diarrhea but not neutropenia was found to be associated significantly with the *UGT1A1* promoter polymorphism [15]. The different irinotecan dosing schedules and combination chemotherapy regimen would probably lead to their impact on the various degrees and severity of drug toxicities, as well as the subsequent analyzing results.

Furthermore, the frequency of neutropenia and diarrhea in Caucasian patients is significantly higher (approximately three- to five-fold) than that in our patients, despite the frequency of nausea/vomiting being not statistically different. Prevalence of the 7TA/7TA genotype is very high in Caucasians (10% of the population) and in African people (20–25%) [15, 28]; conversely, in Asian populations [20, 21, 26], the 7TA/7TA genotype is quite rare, a finding that is in keeping with the results of the current study. From the above findings, we suggest that the relatively lesser presence of 6TA/7TA and 7TA/7TA genotypes may probably play a crucial role in the lower frequency of irinotecan-induced toxicities encountered in Taiwanese mCRC patients. Otherwise, in the current study, the heterozygous or homozygous *UGT1A1*28* seemed to be associated with increased clinical benefit and tumor response. Toffoli *et al.* also pointed out that heterozygous 6TA/7TA and homozygous 7TA/7TA mCRC patients had a significantly reduced risk of stable diseases or progressive diseases compared with the wild-type genotype [18].

Due to the limited case numbers from these studies and variable pharmacodynamics of irinotecan, the available data can only really provide for an estimate of adverse risk effects in patient subpopulations rather than any predictable risk-benefit for an individual patient undergoing treatment (http://www.cdc.gov/genomics/gtesting/EGAPP/about.htm; accessed in May 2009). Therefore, larger mCRC cases to definitely establish the clinical relevance of *UGT1A1* gene polymorphisms on the occurrence of severe toxicities are mandatory. Another important issue is that such genetically-based reduction in irinotecan dosage may result in diminished tumor responsiveness and cancer-specific outcomes since there are significantly higher response rates to standard irinotecan dosing in cases where the phenotype would predict for the highest rate of adverse events [18, 29]. Hence, a careful consideration before irinotecan dose reduction in patients carrying the polymorphic TA_7 allele is recommended. The only way to resolve this issue is to conduct a prospective randomized controlled trial which examines both reduced irinotecan dosage in patients with CRC based on their *UGT1A1* genotype as well as the opposite side of the coin of dose escalation amongst wild-types individuals to improve tumor responsiveness with minimal effects on adverse drug events.

CONCLUSION

Taiwanese mCRC patients with heterozygous or homozygous *UGT1A1*28* have a higher incidence of irinotecan-related severe toxicities. Therefore, determination of the *UGT1A1* genotypes may be potentially beneficial for identifying our patients at-risk of developing a severe or potentially life-threatening toxicity after FOLFIRI regimen.

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