UGT1A1 genotype-specific phase I and pharmacokinetic study for combination chemotherapy with irinotecan and cisplatin: a Saitama tumor board study

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Summary

Introduction: Genotyping of UGT1A1 could be useful for prediction of severe toxicities for patients treated with irinotecan; however, genotype-based recommended dose (RD) has not been established. The aim of the present study was to determine the RD of irinotecan in combination with cisplatin (CPT-P) for individuals with or without UGT1A1 polymorphisms. *Materials and Methods:* According to polymorphisms of UGT1A1*28, *6, and *27, RDs were determined by three-case cohort methods for patients with wild-type and heterotype, and by inter-patient dose escalation for homotype patients. Pharmacokinetic studies were also evaluated. During May 2009 and July 2011, 18 Japanese patients were enrolled; 16 patients with ovarian carcinoma, and two cases with cervical cancer. The RD of irinotecan was determined as 50 mg/m^2 for the patients with wild-type, 40 mg/m^2 for those with heterotype, and 30 mg/m^2 for homotype UGT1A1 genotype. *Results:* Patients with homotype UGT1A1 alleles had a significantly lower glucuronidation ratio in comparison with UGT1A1 wild-type and heterotype cases. *Conclusion:* UGT1A1 genotype-based RDs of irinotecan in CPT-P therapy were determined. Further studies to investigate efficacy of the RD including response evaluation are needed to confirm the present results.

Key words: UDP-glucuronosyltransferase 1A1 (UGT1A1); UGT1A1*6; UGT1A1*28; Irinotecan; Cisplatin; Phase I.

Introduction

Irinotecan hydrochloride is widely-used for a multiplicity of carcinomas, including colorectal and lung cancers. Combination therapy with irinotecan and platinum is often used for not only relapsed gynecologic cancers [1, 2], but also for untreated clear cell carcinoma of the ovary [3-5]. The efficacy of this therapy is currently being explored in the worldwide prospective randomized trial GCIG/JGOG 3017 to compare the survival of patients with ovarian clear cell carcinoma treated with either combination with paclitaxel and carboplatin or therapy with irinotecan and cisplatin [6].

Irinotecan-induced severe toxicities in patients with UGT1A1*28 allele have been reported in several studies [7-11]. A meta-analysis including ten sets of patients demonstrated that the risks of grades 3 and 4 hematologic toxicities were higher among UGT1A1*28/*28 patients than those with UGT1A1*1/*1 and UGT1A1*1/*28 genotype, when treated at medium- or high-dose of irinotecan (> 150 mg/m^2) [12]. The significance was not observed in low-dose of irinotecan (100-125 mg/m^2), which was commonly used at a therapeutic range for weekly and bi-weekly regimens. However, the effects might be potentially influenced by heterogeneity of patients and treatment schedules such as additive use of platinum agents. Another meta-analysis revealed that risk ratio (RR) of severe neutropenia was significantly increased in not only homozygous UGT1A1*28 cases (RR = 3.51), but also in heterozygous cases (RR = 1.82) [13]. The data suggested that the dose of irinotecan should be optimized according to three UGT1A1*28 genotypes; wild-type, heterozygous type, and homozygous type.

In the Asian population, allele frequency of UGT1A1*28 is quite low and another polymorphism of UGT1A1, UGT1A1*6 is more frequently observed compared with Caucasians or Afro-Americans [14, 15]. Recent studies have shown the significant relevance of UGT1A1*6 to severe toxicities of irinotecan-based chemotherapy [16-18].

In the present study, the authors performed UGT1A1 genotype-based phase I study for gynecologic cancer patients treated with weekly irinotecan combined with cisplatin to determine the maximal tolerated dose (MTD) and the recommend dose (RD), and analyzed the pharmacokinetics of irinotecan and its metabolites.

Materials and Methods

Eligibility criteria

The patients were considered to be eligible if they satisfied the following criteria: histologically confirmed diagnosis of ovarian or uterine cervical carcinoma; age range between 20 and 75 years; a performance status between 0 and 2 on the Eastern Cooperative Oncology Group (ECOG) scale; a life-expectancy of at least three months; treatment-free period of at least four weeks from previous chemotherapy or irradiation; adequate hematological (total white blood cell count ≥ 3,000/μl; absolute neutrophil count [ANC] ≥ 1,500/μl; platelet count ≥ 100,000/μl; and hemoglobin level ≥ 9 g/dl), hepatic (total bilirubin level ≤ 1.5 mg/dl; aspartate aminotransferase [AST] and alanine aminotransferase [ALT] levels ≤ three times the upper limit of normal), and renal (creatinine level ≤ 1.5 mg/dl and/or creatinine clearance ≥ 60 ml/minute)

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function. The protocol included the following exclusion criteria: massive ascites and/or massive pleural effusion; serious infectious diseases or other complications, such as uncontrollable diabetes, intestinal pneumonitis, bowel obstruction, active bowel bleeding or colitis, active concurrent malignancies, symptomatic brain metastasis, nursing or pregnant women, medical record of hyperreaction to irinotecan or platinum agents, or other medical problems severe enough to prevent compliance with the present protocol. The study protocol was approved by each institutional review board. All study participants approved informed consent prior to the enrollment of the study.

UGT1A1 genotyping

Serum samples of the patients enrolled in the study were analyzed for polymorphisms of UGT1A1 by using the Invader UGT1A1 Molecular Assay which enabled genotyping of UGT1A1*28, *6, and *27 [19]. UGT1A1 polymorphisms were categorized into three groups; wild-type, heterotype, and homotype. Patients with heterotype UGT1A1 included UGT1A1*28 (*1/*28), UGT1A1*6 (*1/*6), and UGT1A1*27 (*1/*27), and those with no polymorphisms of UGT1A1*28, *6, *27 were categorized as wild-type (*1/*1) group. The cases with homozygous genotype of either UGT1A1*28 (*28/*28) or UGT1A1*6 (*6/*6), and those with double heterozygous polymorphisms of both UGT1A1*28 and UGT1A1*6 (*28/*6) were regarded as homotype patients.

Drug administration

The enrolled patients received chemotherapy consisting of 90-min intravenous infusion of 30 - 70 mg/m^2 of irinotecan on days 1, 8, and 15 and subsequently 120-min intravenous infusion of 60 mg/m^2 of cisplatin on day 1, q4 weeks. Treatment with irinotecan was withheld on days 8 or 15 if the patient experienced hematologic toxicities more than grade 3 or non-hematologic toxicities more than grade 2. Subsequent cycle of the therapy was initiated if the patients showed adequate hematological, hepatic, and renal function as was described in the criteria of patient enrollment, and also no sign of exclusion criteria. The prophylactic use of granulocyte colony-stimulating factor was not allowed.

Starting dose of irinotecan was 50 mg/m^2 for wild-type group patients, 40 mg/m^2 for heterotype patients, and 30 mg/m^2 for homotype patients, respectively. Dose of irinotecan was increased in 10 mg/m^2 increments, unless MTD was achieved. At least three patients were treated at each dose level as shown in Table 1. The patients with wild-type and heterotype were evaluated with three-case cohort methods. If no dose-limiting toxicity (DLT) was observed in the first three patients with wild-type/hetero-type, the dose was escalated at next dose level when more than two patients experienced any DLT, the dose was defined as MTD. When one of three patients developed any DLT, additional three patients were added to the same dose level. If none or only one of additional three cases developed DLT, the dose level was escalated; however, if more than two patients experienced any DLT, the dose was determined as MTD. The RD was defined as one dose level under the MTD. Homotype patients were analyzed by inter-patient dose escalation. Three homotype patients were treated with step 1 dose: 30 mg/m^2 of irinotecan and 60 mg/m^2 of cisplatin. If a patient developed no DLT, the patient would subsequently receive step 2 doses at the second cycle of the therapy. When two or more patients experienced any DLT, the dose was determined as MTD. The RD was defined as one dose level under the MTD.

Toxicity profiles and statistical analysis

Physical examination and serum blood test were carried out at least days 1, 3, 8, 15, and 21 for toxicity evaluation. The toxicity profiles were determined by National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. DLTs were defined as follows; (a) grade 4 neutropenia or leucopenia, (b) grade 3 febrile neutropenia for more than three days, (c) grade 3 non-hematologic toxicities including diarrhea, (d) discontinuation of irinotecan on days 8 and 15 due to toxicities at the first cycle, and (e) delay of the second cycle beyond two weeks due to toxicities.

Pharmacokinetic evaluation

A pharmacokinetics study of irinotecan, SN-38, SN-38 glucuronide (SN-38G), and platinum was performed on day 1 of combination with irinotecan and cisplatin. Whole blood samples were collected at 0, 2, 4, 8, and 24 hours after completion of irinotecan infusion. Irinotecan and its metabolites concentration was measured by high-performance liquid chromatography as described previously [20]. The area under the plasma time-concentration curve (AUC) was calculated using a trapezoidal method. The degree of glucuronidation of SN-38 to SN-38G

Table 1. — *Patients' characteristics.*

UGT1A1 genotype	Wild-type		Heterotype		Homotype	
Dose level	1	2	1	2	1	2
Total no. of patients	3	3	6	3	3	
Ovarian cancer	3	3	5	2	3	
Cervical cancer	0	0	1	1	0	
Age (years)						
Median	56	60	59	53	47	
Range	53-57	59-65	41-66	47-57	33-66	
Weight (kg)						
Median	48	55	54	55	51	
Range	46-50	40-70	35-61	50-79	49-59	
Performance status (ECOG)						
0	3	3	5	3	2	
1	0	0	1	0	1	
Total bilirubin level (mg/dl)						
Median	0.4	0.6	0.5	0.5	0.6	
Range	0.4-0.6	0.4-0.7	0.3-0.7	0.3-0.7	0.5-0.8	
Prior chemotherapy						
Yes	2	3	6	2	2	
No	1	0	0	1	1	
Prior radiotherapy						
Yes	0	0	1	1	0	
No	3	3	5	2	3	

ECOG = Eastern Cooperative Oncology Group.

Table 2. — *Dose-escalation scheme and incidence of dose-limiting toxicity (DLT).*

Dose level	Irinotecan (mg/m^2)	Cisplatin (mg/m^2)	Number of patients	Number of DLT
Wild-type (*1/*1)				
1	50	60	3	0
2	60	60	3	2 (ANC, FN)
Heterotype (*28/*1, *6/*1)				
1	40	60	6†	1† (ANC)
2	50	60	3	2 (diarrhea, diarrhea + FN)
Homotype (*28/*28, *6/*6, *28/*6)				
1	30	60	3	0
2	40	60	3	2 (ANC + FN, ANC + ALT)

† Three additional patients were treated to confirm the feasibility of the dose level; ANC = neutrophil count decreased; FN = febrile neutropenia; ALT = alanine aminotranseferase increased.

Table 3. — *Pharmacokinetic parameter at the first cycle*.

UGT1A1 genotype dose level	Irinotecan AUC (ng*h/ml)	SN-38 AUC (ng*h/ml)	SN-38G AUC (ng*h/ml)	GR	Platinum AUC (μg*h/ml)
Wild-type					
Step 1 (n = 3)	2,329 ± 121	46.5 ± 0.5	524 ± 214	11.2 ± 3.3	89.5 ± 15.5
Step 2 (n = 2)	4,574 ± 721	120 ± 28	779 ± 144	6.5 ± 1.0	92.2 ± 21.1
Heterotype					
Step 1 (n = 6)	2,881 ± 307	55.3 ± 10.8	516 ± 197	9.8 ± 2.9	80.6 ± 16.7
Step 2 (n = 2)	3,107 ± 654	107 ± 21	873 ± 46	8.1 ± 1.4	75.5 ± 20.4
Homotype					
Step 1 (n = 2)	1,730 ± 665	76 ± 37	336 ± 78	3.4 ± 0.6†	68.9 ± 19.3
Step 2 (n = 2)	1,650 ± 528	136 ± 25	226 ± 36	2.4 ± 0.8†	70.5 ± 16.9

†GR of homotype patients was significantly lower than that of wild-type/heterotype patients: 3.1 vs 8.7, $p = 0.001$ (Mann-Whitney U-test). AUC = area under the plasma concentration curve; GR = glucuronidation ratio = [AUC of SN-38G]/[AUC of SN-38].

(Glucuronidation ratio; GR) was defined as the ratio of SN-38G AUC/ SN-38 AUC. Total platinum and filterable platinum were measured using the same whole blood samples.

Estimated values of the pharmacokinetic parameters were reported as mean ± SE. Student's *t*-test or Mann-Whitney U-test was used for statistical analysis by the Stat View software ver. 5.0 (SAS Institution Inc., Cary, NC, USA). A p value of < 0.05 was considered statistically significant.

Response evaluation

Response was evaluated with computed tomography (CT) or magnetic resonance imaging (MRI) after two cycles of chemotherapy in patients with measurable disease. Tumor response was assessed using Response Evaluation Criteria in Solid Tumors [21]. Responses were confirmed by CT at least four weeks later. Response evaluation of chemotherapy was not done by serum levels of CA125 in patients with ovarian carcinoma in the present study.

Results

Patients' characteristics

During May 2009 and July 2011, 18 Japanese patients (patients) were enrolled; 16 ovarian carcinoma patients, and two cervical cancers. The patients' characteristics according to each dose level are listed in Table 1. Genotype of UGT1A1 was wild-type in six patients (33%), heterotype in nine patients (50%), and homotype in three patients (17%). Heterotype included four patients of *28, five patients of *6, and no cases of *27. Genotypes of three patients with homotype were *6/*6, *28/*28, and *6/*28. Median age was 55 years, ranging from 35 to 66 years. Among 18 cases enrolled, 15 cases (83%) had received previous chemotherapy, and two cervical cases (11%) had undergone pelvic irradiation. There were no significant differences of total bilirubin levels among UGT1A1 genotypes.

Dose-escalation results

A summary of dose-escalation schema are shown in Table 2. At dose level 1 for wild-type cases, there were no DLTs in all three cases. At dose level 2 of wild-type cases, two of three cases developed DLTs: a case with grade 4 neutropenia, and another with grade 3 febrile neutropenia. Dose level 2 was defined as the MTD for wild-type cases. RD for wild-type cases was dose level 1, which used irinotecan at a dose of 50 mg/m^2.

At dose level 1 for heterotype cases, one of three cases had grade 3 neutropenia over 25 days and developed DLT. There were no DLTs in additional three cases. At dose level 2 for heterotype cases, DLTs were observed in two of three cases. One developed grade 3 diarrhea, and the other had both grade 3 febrile neutropenia and grade 4 diarrhea. Dose level 2 was determined as MTD, and RD was dose level 1.

Three cases with homotype had no DLTs at dose level 1. However, two patients developed DLTs at dose level 2: a case with grade 4 neutropenia and grade 3 febrile neutropenia, and another with grade 4 neutropenia and grade 3 non-hematologic toxicity of increased alanine aminotranseferase. Dose level 2 was determined as MTD, and RD was dose level 1 for homotype patients.

RD of weekly irinotecan was 50 mg/m^2 for UGT1A1 wild-type patients and 40 mg/m^2 for hetero-type, and 30 mg/m^2 for homotype UGT1A1 patients.

Pharmacokinetics of irinotecan, SN-38, SN-38G, and platinum

Pharmacokinetic parameter at each dose level is shown in Table 3. AUCs of SN-38 were higher in step 2 in comparison with step 1 doses in all three groups. GR of homotype patients was significantly lower than that of wild-type/heterotype patients: 3.1 vs 8.7, $p = 0.001$ (Mann-Whitney U test). There were no significant differences of AUCs of platinum among three groups, and between two steps.

Response evaluation

As the present study was a dose-finding phase I study, primary endpoint did not include evaluation of response. In response evaluable cases, objective response was observed in one of the two wild-type dose level 1, one in two wild-type dose level 2, zero in five heterotype dose level 1, zero in three heterotype dose level 2, and one in two homotype patients.

Discussion

There have been reports of more than 100 polymorphisms in the UGT1A1 gene [22]. A UDP-glucuronosyltransferase (UGT) polymorphism, UGT1A1*6, is one of single nucleotide polymorphisms (SNPs) locating on the exon1 coding region of UGT1A1 gene, which was rarely seen in Caucasians but negligible in clinical studies using Asians populations. In the present study, the authors observed six cases (33%) with heterotype UGT1A1*6 allele, and one case (6%) with homotype UGT1A1*6 alleles, supporting higher abundance of UGT1A1*6 polymorphism in the Asian population. Previous report described the significant increase of basal level of serum total bilirubin in patients with UGT1A1*28 or UGT1A1*6 [17]. In the present cases, serum level of total bilirubin was not significantly elevated in homotype patients in comparison with wild-type or heterotype cases, which potentially sug-

gested that it would be difficult to estimate UGT1A1 genotypes according to serum level of total bilirubin.

The present dose-finding phase I study revealed that RDs of irinotecan in combination with 60 mg/m^2 of cisplatin for phase II/III studies were 50 mg/m^2 for UGT1A1 wild-type patients, 40 mg/m^2 for hetero-type patients, and 30 mg/m^2 for homotype patients. DLTs included neutropenia, febrile neutropenia, diarrhea, and increased alanine aminotransferase, which were common toxicities in patients treated with irinotecan and cisplatin. In patients with homotype UGT1A1 alleles, significantly lower levels of GR were observed in comparison with wild-type and heterotype genotypes. Also, AUCs of SN-38 were higher than those with wild-type and heterotype genotypes. These results suggested that UGT1A1 genotype is needed even for patients treated with relatively lower dose (~125 mg/m^2) of irinotecan. Previous report revealed that a significant predictor of grade 3/4 neutropenia or diarrhea was previous pelvic radiotherapy [18]. Actually, the present study included two cervical cancer cases treated with pelvic radiotherapy: a case treated with heterotype dose level 1 (no DLT), and another treated with heterotype dose level 2 (DLT of diarrhea). The development of DLT in the second case suggested that the previous pelvic radiotherapy could potentially lead to severe toxicities with a combination of irinotecan and cisplatin. Additionally, ECOG PS and a number of present chemotherapeutic regimens were not related to severe toxicities in the present study. From the AUCs of platinum observed in the present study, there were no significant differences of platinum concentration. So the authors would strongly recommend the genotyping of UGT1A1 for patients that will be treated with irinotecan and platinum.

There might be other factors influencing pharmacokinetics of irinotecan-based chemotherapy. However, the present study demonstrated the significant association of genotyping of UGT1A1*6 and *28 in patients treated with cisplatin and low dose of irinotecan. In the clinical settings, genotyping of UGT1A1*6 in addition to UGT1A1*28 would be recommended in the patients treated with irinotecan and cisplatin, especially in the Asian population. Also, the authors recommend these RDs determined in the present study be evaluated in further phase II / III studies.

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