SNP Communications

Novel Single Nucleotide Polymorphism of UGT1A7 Gene in Japanese

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Summary: We sequenced exon 1 of the UDP-glucuronosyltransferase (UGT) 1A7 gene from 52 Japanese cancer patients. Four single nucleotide polymorphisms (SNPs) were found. Three of them caused UGT1A7*2 and UGT1A7*3. A novel SNP (98973G>C) causing amino acid substitution (Ser141Cys) was found. The sequence is as follows: SNP, 050824FujitaK002; GENE NAME, UGT1A7; ACCESSION NUMBER, AF297093; LENGTH, 25 bases; 5’-TAAAGGAGAGTTGC-3’.

One out of 52 cancer patients was heterozygous for the variant allele, resulting in the allele frequency of 0.96%. The patient did not possess UGT1A7*2 or UGT1A7*3.

Key words: UGT1A7; novel SNP; Japanese; Irinotecan

Introduction

UDP-glucuronosyltransferase (UGT) is one of phase II drug-metabolizing enzymes that catalyzes the glucuronidation of a wide variety of endogenous and exogenous compounds, including drugs, carcinogens, and other xenobiotics. The UGT superfamily is composed of families, and two of the families (UGT1 and UGT2) have the ability to catalyze the glucuronidation of foreign chemicals. The UGT1A7 gene located on chromosome 2q37 is expressed exclusively in the oropharynx, esophagus, stomach and pancreas, but is absent from the liver. Cloning and characterization of the UGT1A7 gene product revealed the glucuronidation of known carcinogens, which included tobacco smoke carcinogens. Chemicals with phenolic, anthraquinone, flavone, coumarin and naphthol structures are substrates for UGT1A7. UGT1A7 is also known to be involved in the glucuronidation of an active metabolite of an anticancer drug irinotecan (SN-38). Various single nucleotide polymorphisms (SNPs) have been identified in the UGT1A7 gene so far. UGT1A7*3 has been known to cause the reduced capacity to metabolize SN-38, whereas the product of UGT1A7*2 gene showed higher activity toward SN-38 than that of UGT1A7*1. The effects of these polymorphisms on the irinotecan toxicity in humans seem somewhat contradictory. Carlini et al. claimed that the low UGT1A7 activity was related to the little toxicity of irinotecan, whereas Ando et al. demonstrated no relationship between the reduced UGT1A7 activity and the irinotecan toxicity.

In the present study, the exon 1 of UGT1A7 gene from 52 Japanese cancer patients treated with irinotecan was sequenced, and found a novel SNP causing amino acid substitution.

Materials and Methods

Patients: Fifty-two Japanese cancer patients (32 colorectal cancer, 15 gastric cancer, 5 others) who received various regimens of irinotecan-containing chemotherapy or irinotecan monotherapy from June 2003 to August 2005 were studied. All of patients gave informed consent in writing for their peripheral blood samples and medical information to be used for the research. This study was approved by the Institutional Review Board of Saitama Medical School.

Human DNA samples: Genomic DNA was extracted from 200 μL of peripheral blood of 52 Japanese
cancer patients, which had been stored at −80°C until analysis, by using QIAamp Blood Kit (QIAGEN GmbH, Hilden, Germany).

**PCR conditions and DNA sequencing:** Exon 1 of the UGT1A7 gene was analyzed by direct sequencing of a PCR product by the method of Carlini et al.11, with minor modifications. Briefly, the reaction mixture for PCR consisted of 2.5 mM MgCl₂ and 1.25 unit of AmpliTaq Gold polymerase (Perkin-Elmer, Foster City, CA) in a final volume of 50 μL. The sequence of the complete UGT1A7 gene described in the GeneBank (AF297093) was used as a reference.

Two polymorphisms of UGT1A1 gene (UGT1A1*6 and UGT1A1*27) were determined by PCR-RFLP method as described by Ando et al.,15 with minor modifications. Briefly, the first and the second PCRs were performed with AmpliTaq Gold. The reaction mixture for the first PCR consisted of 2 mM MgCl₂ and 1.25 unit of AmpliTaq Gold polymerase in a final volume of 50 μL. The second PCR was carried out with the reaction mixture consisted of 1.5 mM MgCl₂ and 1.25 unit of AmpliTaq Gold polymerase in a final volume of 50 μL. The PCR conditions for the second PCR were: 95°C 15 min followed by 25 cycles of 94°C for 30 s, 55°C for 40 s and 72°C for 40 s. RFLP analyses for UGT1A1*6 and UGT1A1*27 were performed with Msp I (Takara, Otsu, Japan) and Bsr I (New England Biolabs, Ipswich, MA), respectively. The TATA box polymorphism (UGT1A1*28) was determined by the direct sequencing described by Ando et al.,15 with minor modifications. Briefly, the reaction mixture for the first PCR consisted of 1 mM MgCl₂ and 1.25 unit of AmpliTaq Gold polymerase in a final volume of 50 μL. The PCR conditions for the first PCR were: 95°C 15 min followed by 30 cycles of 95°C 30 s, 58°C 40 s and 72°C 40 s.

**Treatments:** Irinotecan as a monotherapy was given weekly at a dose of 100 mg/m² for the first 3 weeks of each 4-week cycle,16 or biweekly at a dose of 150 mg/m²17 until the disease showed progression or intolerable toxicity occurred. As combination chemotherapy, a 100 mg/m² irinotecan was administered with the bolus 5-fluorouracil (FU) 500 mg/m² and leucovorin (LV) 10 mg/m² (l isomer form) weekly for the first 4 weeks of each 6-weeks cycle (IFL regimen).18 The FOLFIRI regimen administered at 2-week intervals comprised irinotecan at escalating doses from 150 to 180 mg/m² and LV 200 mg/m² administered over 2 hours followed by FU 400 mg/m² as a bolus injection and FU 2,400 mg/m² as a 46-hour continuous infusion.19 As the other regimen repeated every 4 week (IP regimen), irinotecan at doses from 50 to 70 mg/m² was followed 2 hours later by a 120-minute infusion of cisplatin 80 mg/m² with adequate hydration on day 1, and the same dose of irinotecan was repeated on day 15.20 In each regimen, irinotecan at doses from 50 mg/m² to 180 mg/m² dissolved in 250 mL of 5% dextrose solutions was infused over 90 minutes.

**Pharmacokinetic analysis:** Blood sampling for pharmacokinetic analysis was typically performed at their first courses. The blood samples for the analysis were taken from the arm opposite the infusion site at the beginning of irinotecan infusion and 0, 0.25, 0.5, 1, 2, 4, 8, and 24 hours after the end of the infusion. Plasma was immediately obtained by centrifugation of the blood samples and stored at −80°C until analysis.

Total (lactone and carboxylate) plasma concentrations of irinotecan, SN-38 and SN-38 glucuronide (SN-38G) were analyzed at the institution, using a reverse-phase HPLC method. A 150 μL of plasma sample was mixed with 300 μL of methanol, 5% perchloric acid (50:50, v/v) and camptothecin as an internal standard by a vortex mixer. The 200 μL of supernatant obtained by centrifugation of the mixture at 15,000 rpm for 10 min was injected into a HPLC system (Hitachi model 7000 series, Hitachi, Tokyo, Japan) equipped with a TSK-gel ODS-120T analytical column (4.6×250 mm; 4 μm; TOSOH, Tokyo, Japan), and separated at 40°C, at a flow rate of 1.0 mL/min to quantify the total (lactone and carboxylate) plasma concentrations of irinotecan, SN-38 and SN-38G. The mobile phase consisted of 75 mM ammonium acetate (pH 4.75) for solvent A and acetonitrile for solvent B: a 20-minute run of a linear gradient of 85% to 65% of solvent A. Lower limit of quantification for irinotecan was 5 ng/mL, and those for SN-38 and SN-38G were 0.5 ng/mL. The intra- and inter-assay coefficient variations for irinotecan and the metabolites were under 10%.

Area under the time versus concentration curve (AUC, μmol·h/l) from the beginning of the infusion to the last sampling was calculated by the linear trapezoidal rule, using a computer program, WinNonlin version 4.01 software (Pharsight Corporation, Mountain View, Calif).

**Results and Discussion**

We found a following novel SNP: SNP, 050824Fujita002; GENE NAME, UGT1A7; ACCESSION NUMBER, AF297093; LENGTH, 25 bases; 5’-TAAAGGAGATTTGCCTTTGTAATGCAGT-3’. The SNP was 98973G>C in exon 1 of UGT1A7 (Fig. 1), resulting in the amino acid substitution (Ser141Cys). This novel SNP was found in one Japanese colorectal cancer patient with a heterozygosity. Thus, the allele frequency was calculated to be 0.96%. The patient possessing the novel SNP did not harbor UGT1A7*2 or UGT1A7*3 variant in exon 1, suggesting the no linkage among these SNPs.

Although functional significance of this SNP has not been known, it may cause functional reduction of
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Fig. 1. Nucleotide sequences of UGT1A7 containing novel variant 050824FujitaK002 (98973G Â C) in exon 1. Arrows indicate the positions of the nucleotide change.

UGT1A7 because the amino acid of 141 is located in the putative substrate-binding domain of the enzyme (between Ser<sup>141</sup> and Phe<sup>150</sup>). The idea is considered to be supported by the fact that the decrease of the enzymatic activity of UGT1A7 by the known amino acid substitutions in the putative substrate-binding domain (UGT1A7.3).

UGT1A7 plays a role in the glucuronidation of the active metabolite of irinotecan (SN-38). Therefore, patient with reduced activity of UGT1A7 may have decreased ability to detoxify the SN-38. Besides UGT1A7, UGT1A1 is known to be responsible for the detoxification of SN-38, and the polymorphisms in the UGT1A1 gene such as UGT1A1*28, UGT1A1*6 and UGT1A1*27 have been demonstrated to be related to the enhanced toxicity of irinotecan such as diarrhea and neutropenia. The analysis of the UGT1A1 polymorphisms for the patient with the novel SNP revealed that the patient harbored the wild type of UGT1A1 gene. Therefore, to clarify the effects of the novel SNP in UGT1A7 gene on the glucuronidation capacity for SN-38, pharmacokinetics in SN-38 and SN-38G were compared between the patient with the novel SNP (not possessing UGT1A7*2 or UGT1A7*3 and UGT1A1*28, UGT1A1*6 or UGT1A1*27) and patients without all of these SNPs in UGT1A1 and UGT1A7 genes. According to the genetic analysis, 16 patients possessed the wild type of UGT1A1 and UGT1A7. The AUC ratio of SN-38 to SN-38G seen in the patient with the novel SNP (0.42) was not necessarily higher than those observed in the 16 patients (0.38 to 0.57; quartile range). In addition, the patient possessing the novel SNP did not show severe toxicity by the irinotecan treatment. Accordingly, heterozygosity of the novel SNP appeared not to affect the pharmacokinetics of irinotecan metabolites and the toxicity of irinotecan. The functional significance of the novel SNP in the UGT1A7 gene is needed to be examined in the future.

In conclusion, we found a novel nonsynonymous mutation 98973G Â C in exon 1 of UGT1A7 in a DNA sample from a Japanese colorectal cancer patient. The SNP caused the amino acid substitution (Ser<sup>141</sup>Cys).

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