Cancer Chemother Pharmacol (2009) 63:1165–1169
DOI 10.1007/s00280-008-0864-x

SHORT COMMUNICATION

Life-threatening toxicities in a patient with *UGT1A1*6/*28* and *SLCO1B1*15/*15* genotypes after irinotecan-based chemotherapy

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Received: 28 May 2008 / Accepted: 19 October 2008 / Published online: 8 November 2008

Abstract
Introduction To explore severe toxicities induced by irinotecan-based chemotherapy and *UGT1A1*6/*28* and *SLCO1B1*15/*15* genotypes.
Case report A 66-year-old Japanese male diagnosed with left pharyngeal carcinoma (T2N2bM0, stage IVA) was treated with irinotecan (70 mg/m^2) on days 1, 8 and 15 in combination with docetaxel (60 mg/m^2) on day 1 of a 28-day cycle. After the first cycle, he suffered marked toxicities, including grade 4 diarrhea and febrile grade 4 neutropenia. Plasma concentrations of irinotecan, SN-38 and SN-38G were measured, and extensive accumulation of SN-38 was observed. Genotyping of UGT1A1 and OATP1B1 proteins showed *UGT1A1*6/*28* and *SLCO1B1*15/*15*, respectively, which are known to lead to extremely low glucuronidation and transport activities of substrate drugs.
Conclusion The severe toxicities in this patient are attributable to the extensive accumulation of SN-38, which may result from a synergistic or additive effect of low metabolic (*UGT1A1*6/*28*) and transport (*SLCO1B1*15/*15*) capabilities.

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Keywords Irinotecan · Pharmacokinetics · Toxicity · Pharmacogenetics · *UGT1A1* · *SLCO1B1*

Introduction

Irinotecan (CPT-11) is metabolized by carboxylesterases to form an active SN-38, which is further conjugated to an inactive glucuronic acid conjugate (SN-38G) by UDP-glucuronosyltransferase 1A1 (UGT1A1) [1, 2]. Large interindividual variability in the pharmacokinetics of SN-38 is likely to be associated with severe toxicities, such as neutropenia and delayed-type diarrhea [3–5]. Polymorphism of the *UGT1A1* gene is known to play an important role in irinotecan pharmacokinetics and severe toxicities. *UGT1A1*28*, characterized by an extra seventh dinucleotide (TA) insertion in the $(TA)_6$TAA-box in the promoter region, leads to decreased glucuronidation of SN-38; thus, genotyping of **28* allele could help to predict irinotecan-associated toxicity [6, 7]. In addition, *UGT1A1*6* (211G>A) variant, highly observed in the Asian population, is associated with a reduction in SN-38 glucuronide formation [8–10]. Organic anion transporting polypeptide 1B1 (OATP1B1, gene *SLCO1B1*), expressed on the basolateral membrane in hepatocytes, has been reported to contribute to the hepatic uptake of SN-38 [11]. Recent reports suggest that the *SLCO1B1*15* haplotype (388A>G and 521T>C) results in decreased uptake activity of SN-38, leading to increased plasma concentration of irinotecan and SN-38 [12–14]. Here, we report the case of a patient homozygous for *SLCO1B1*15* and compound heterozygous for *UGT1A1*6* and **28*, who suffered from marked accumulation of SN-38 after irinotecan-based chemotherapy, resulting in life-threatening toxicities.

Case report

The patient was a 66-year-old Japanese male who came to the hospital after becoming aware of a left neck mass, and left hypopharyngeal squamous cell carcinoma (T4N2bM0, stage IVA) was diagnosed. He received 5-fluorouracil-based chemotherapy and radiotherapy. The primary hypopharyngeal carcinoma responded well to chemoradiotherapy. Eight months later, he received combination chemotherapy of irinotecan and docetaxel, because a recurrence of neck metastasis was detected by computed tomography (CT) scan. Pretreatment laboratory tests revealed total leukocyte count, 4,800/μL, absolute neutrophil count 4,080/μL, platelet count 253 × 10^3/μL, hemoglobin 12.0 g/dL, total bilirubin 1.0 mg/dL, serum aspartate aminotransferase (AST) 24 IU/L, serum alanine aminotransferase (ALT) 27 IU/L, alkaline phosphatase (ALP) 417 IU/L, and serum creatinine (Cr) 0.61 mg/dL. He had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 and received only oral famotidine 20 mg/day for at least one month. In the first cycle, irinotecan at a dose of 70 mg/m^2 on days 1, 8 and 15 in combination with docetaxel at a dose of 60 mg/m^2 on day 1 were administered in the absence of premedication to reduce irinotecan-induced delayed diarrhea. After the first cycle, he suffered marked toxicities, including grade 4 watery diarrhea (10–18 times/day) on day 8–10, for which he was treated with loperamide (2 mg/day) on day 10, and febrile grade 4 neutropenia (nadir 36/μL) on day 9 according to the Common Terminology Criteria for Adverse Events, Version 3.0 (CTCAE v3.0). Electrolyte disturbance followed by stupor due to severe diarrhea occurred, for which he was administered fluids and electrolytes parenterally. Moreover, he required the continuous administration of granulocyte-colony stimulating factor and cefepime; therefore, chemotherapeutic treatment on day 8 was discontinued. Despite the adverse effects, he subsequently received a second cycle of this regimen because of the excellent response. In the second cycle, the dose of irinotecan and docetaxel were reduced to 27 and 36 mg/m^2, respectively. Pretreatment laboratory data findings were: total bilirubin 1.2 mg/dL, AST 24 IU/L, ALT 21 IU/L, ALP 338 IU/L, and Cr 0.65 mg/dL. Prior to the second cycle of therapy, his informed consent was obtained for participation in an irinotecan pharmacokinetic and pharmacogenetic study, which was approved by the Ethics Review Board of Tottori University. The patient completed the second cycle of therapy, although he had grade 3 neutropenia with prophylactic granulocyte colony-stimulating factor support, but not diarrhea despite an absence of premedication.

The life-threatening toxicities (diarrhea and febrile neutropenia) observed after the first cycle of irinotecan-based chemotherapy raised the suspicion of a deficiency in drug metabolism. Blood samples were obtained 2, 8 and 23 h after the start of irinotecan infusion (90 min intravenous infusion) on day 1 of the second cycle. Serum concentrations of total irinotecan and its metabolites were measured by high-performance liquid chromatography according to previously described methods [15, 16]. Pharmacokinetic parameters were estimated by the Bayesian method using Klein's population pharmacokinetic parameters as initial parameter sets [17]. In order to explore the pharmacokinetic profiles of this case, we incorporated our previously reported patients as a control [13], who had the *UGT1A1*1/*1* genotype (two were heterozygous for *SLCO1B1*15* allele and two were noncarriers of **15* allele), into Fig. 1.

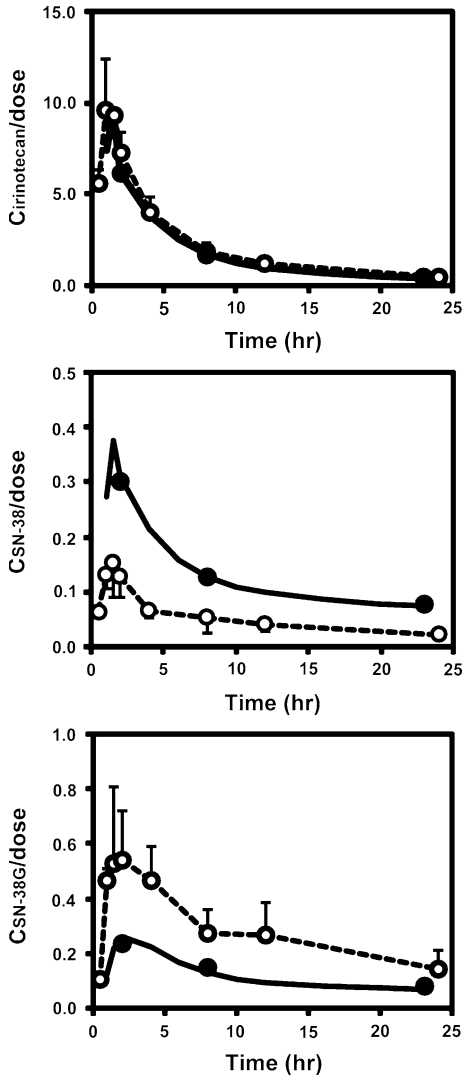

Fig. 1 Serum concentration (normalized by irinotecan dose mg/m^2) profiles of irinotecan and its metabolites after a 90 min intravenous infusion of irinotecan. *Closed circles*, this case (irinotecan dose at 27 mg/m^2); *open circles*, *UGT1A1*1/*1* patients (our previous patients [13], two were heterozygous for *SLCO1B1*15* allele and two were non-carriers of **15* allele) (60–100 mg/m^2, $n = 4$). Each value is expressed as the mean ± SD

Table 1 Effect of *UGT1A1* and *SLCO1B1* genotypes on AUC_{SN-38G}/AUC_{SN-38} ratio in Asian cancer patients [9, 12, 13]

Reference	Genotype		*N*	AUC_{SN-38G}/AUC_{SN-38} ratio
	UGT1A1	*SLCO1B1*		
This case	**6/*28*	**15/*15*	1	0.92
[9]	**1/*1*		55	6.13 (4.72–7.79)[a]
	**1/*6*		32	4.03 (2.74–5.97)[a]
	**6/*6*		5	1.19 (1.06–3.74)[a]
	**1/*28*		26	3.65 (2.76–5.21)[a]
	**28/*28*		4	3.65 (2.05–4.92)[a]
	**6/*28*		7	2.03 (1.65–3.26)[a]
[12]		**1a/*1b*, **1b/*1b*	54	8.91 ± 7.45[b]
		**1b/*15*, **15/*15*	11	3.57 ± 1.95[b]
[13]	**1/*1*	**1a/*1b*, **1b/*1b*, **1a/*15*, **1b/*15*	4	6.16 ± 1.30[b]
	**1/*6*	**1b/*15*	2	2.67, 3.19
	**1/*1*	**15/*15*	1	3.32

[a] Interquartile range

[b] Mean ± SD

The area under the concentration–time curves from time zero to infinity (AUCs) of irinotecan (dose of 27 mg/m^2), SN-38, and SN-38G were 1,405.7, 189.0 and 174.3 ng h/ml, respectively. The AUC ratio of SN-38G to SN-38 ($AUC_{SN-38G/SN-38}$ ratio) was 0.92. SN-38 and SN-38G serum concentrations normalized by the irinotecan dose (27 mg/m^2) in this patient were higher and lower than the corresponding mean values in the control patients (60∼100 mg/m^2, $n = 4$), respectively. Also, SN-38 and SN-38G AUCs normalized by the irinotecan dose (mg/m^2) were 338% higher and 34% lower in this patient (7.0 and 6.5) compared with the mean values in control patients (1.6 ± 0.4 and 9.8 ± 3.7, mean ± SD). Genotyping and haplotyping of the *SLCO1B1*15*, *UGT1A1*6* and **28* were identified by either polymerase chain reaction (PCR)-restriction fragment length polymorphism or direct sequencing analysis, as described previously [13]. Diplotyping of the *UGT1A1*6/*28* was determined using subcloning of PCR fragments containing both alleles and direct sequencing analysis. He was heterozygous for both *UGT1A1*6* and **28*. Additional genotyping analysis indicated that this patient was a homozygous carrier of the *SLCO1B1*15* allele.

Discussion

Our patient experienced life-threatening toxicities involving neutropenia and diarrhea following irinotecan plus docetaxel chemotherapy. Docetaxel is primarily eliminated by a metabolic enzyme, cytochrome P450 (CYP) 3A4 [18]; however, this patient did not receive any medications known to inhibit CYP3A4, and also had neither liver nor renal dysfunction; therefore, the severe toxicities observed in this case could not be explained by CYP3A4-mediated interactions. The $AUC_{SN-38G/SN-38}$ ratio in our patient was lower than the previously reported median value of 2.03 (range 1.65–3.26) or 3.65 (2.05–4.92) in Japanese subjects with *UGT1A1*6/*28* or **28/*28* genotypes, respectively (Table 1), suggesting markedly low SN-38 glucuronidation capacity. Therefore, in our patient, the SN-38 concentration after irinotecan administration (dosage of 70 mg/m^2) in the first cycle can be considered surprisingly high, resulting in severe toxicities. Severe neutropenia has been reported in *UGT1A1*28/*28* patients whose serum SN-38 levels were high [7]. Recent studies indicate that the *UGT1A1*6* allele is associated with low glucuronidation activity and is involved in severe neutropenia. Minami et al. [9] reported severe neutropenia after irinotecan therapy in a patient with *UGT1A1*6/*28* genotype.

Our patient was a homozygous carrier of the *SLCO1B1*15* allele. Nozawa et al. [11] reported that SN-38 is a very good substrate for OATP1B1, which is expressed on the basolateral membrane in hepatocytes, responsible for the hepatocellular uptake of SN-38 from the systemic circulation. Han et al. [14] reported that the *SLCO1B1*15* variant was associated with increased systemic exposure of SN-38 but not irinotecan; similar results were recognized in our patient. In contrast, we [13] and Xiang et al. [12] found that the *SLCO1B1*15* variant led to not only increased SN-38, but also irinotecan systemic exposure. Although the reasons for the discrepancy with their findings are not clear, these findings suggest that low transport activity of OATP1B1 attributable to the *SLCO1B1*15* variant leads to increase systemic exposure to SN-38. A significant relationship between the *SLCO1B1*15* allele and severity of neutropenia has been reported [14]. These studies also showed a low $AUC_{SN-38G/SN-38}$ ratio in patients with the *SLCO1B1*15* allele [12–14], which is probably due to reduction of the hepatocellular

uptake of SN-38 via OATP1B1 in patients with the *SLCO1B1*15* allele. Our patient with *UGT1A1*6/*28* had a lower $AUC_{SN\text{-}38G/SN\text{-}38}$ ratio (0.92) than Japanese cancer patients with the corresponding *UGT1A1* genotype (Table 1). Hence, a synergistic or additive effect of *UGT1A1*6/*28* with *SLCO1B1*15/*15* on hepatic SN-38 disposition may contribute to the excessive accumulation of SN-38, resulting in the severe neutropenia observed in this patient.

Some studies failed to show a significant association between the *UGT1A1*6* and/or **28* variants and diarrhea severity [7–10]. Ando et al. [6] reported that a genotype either homozygous or heterozygous the *UGT1A1*28* is a risk factor for not only severe neutropenia but also diarrhea by irinotecan. On the other hand, Han et al. [14] did not find a significant relationship between the *SLCO1B1*15* allele and severe diarrhea. In contrast, we previously experienced severe neutropenia and diarrhea after irinotecan-based chemotherapy in a patient with *SLCO1B1*15/*15* [13]. Differences in irinotecan dosage, coadministered chemotherapeutic drugs and treatments for diarrhea may contribute to the discrepancy between these polymorphisms and diarrhea severity.

SN-38 is formed from irinotecan in the liver by carboxylesterases, then apparently transported back into the circulation, and then supposedly transported from blood back into hepatocytes by among other transporters, except for OATP1B1. The second step occurs despite competing pathways, including intracellular glucuronidation by multiple UGT isoforms and the occurrence of active efflux of SN-38 on the hepatobiliary surface by multiple ATP-binding cassette transporters [19]. The overall complexity of the SN-38 elimination pathway makes it difficult to comprehend that a single haplotype in a single gene would be associated with such large phenotypic changes. However, Han et al. [20] recently reported that *SLCO1B1* 521T>C variant is independently associated with higher AUC of SN-38 and severe neutropenia among genetic polymorphisms in candidate UGT and transporter genes, suggesting that *SLCO1B1* variant is an important factor for the variability in SN-38 disposition.

The combined genotype of *UGT1A1*6/*28* and the *SLCO1B1*15/*15* may be strongly associated with the excessive accumulation of SN-38, resulting in irinotecan-related severe toxicities. The frequency of homozygotes and compound heterozygotes for *6 and *28 is reported to be about 9% in Japanese [9]. Moreover, the homozygous carrier for *SLCO1B1*15* allele can be observed at extremely low frequency (0.8% in Japanese) [21]. Although this particular combination genotype is very rare, combined genotyping of *UGT1A1*6*, **28* and *SLCO1B1*15* variants may be useful in irinotecan-based chemotherapy to avoid life-threatening toxicities.

Acknowledgments This study is supported by Health and Labor Sciences Research Grants from the Ministry of Health, Labor and Welfare, Tokyo, Japan.

References

1. Kawato Y, Aonuma M, Hirota Y, Kuga H, Sato K (1991) Intracellular roles of SN-38, a metabolite of camptothecin derivative CPT-11, in the antitumor effect of CPT-11. Cancer Res 51:4187–4191
2. Iyer L, King CD, Whitington PF, Green MD, Roy SK, Tephly TR, Coffman BL, Ratain MJ (1998) Genetic predisposition to the metabolism of irinotecan (CPT-11): role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. J Clin Invest 101:847–854
3. Mathijssen RH, van Alphen RJ, Verweij J, Loos WJ, Nooter K, Stoter G, Sparreboom A (2001) Clinical pharmacokinetics and metabolism of irinotecan (CPT-11). Clin Cancer Res 7:2182–2194
4. Chabot GG, Abigerges D, Catimel G, Culine S, de Forni M, Extra JM, Mahjoubi M, Herait P, Armand JP, Bugat R, Clavel M, Marty ME (1995) Population pharmacokinetics and pharmacodynamics of irinotecan (CPT-11) and active metabolite SN-38 during phase I trials. Ann Oncol 6:141–151
5. Kudoh S, Fukuoka M, Masuda N, Yoshikawa A, Kusunoki Y, Matsui K, Negoro S, Takifuji N, Nakagawa K, Hirashima T, Yana T, Takada M (1995) Relationship between the pharmacokinetics of irinotecan and diarrhea during combination chemotherapy with cisplatin. Jpn J Cancer Res 86:406–413
6. Ando Y, Saka H, Ando M, Sawa T, Muro K, Ueoka H, Yokoyama A, Saitoh S, Shimokata K, Hasegawa Y (2000) Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. Cancer Res 60:6921–6926
7. Toffoli G, Cecchin E, Corona G, Russo A, Buonadonna A, D'Andrea M, Pasetto LM, Pessa S, Errante D, De Pangher V, Giusto M, Medici M, Gaion F, Sandri P, Galligioni E, Bonura S, Boccalon M, Biason P, Frustaci S (2006) The role of UGT1A1*28 polymorphism in the pharmacodynamics and pharmacokinetics of irinotecan in patients with metastatic colorectal cancer. J Clin Oncol 24:3061–3068
8. Jada SR, Lim R, Wong CI, Shu X, Lee SC, Zhou Q, Goh BC, Chowbay B (2007) Role of *UGT1A1*6*, *UGT1A1*28* and *ABCG2* c.421C>A polymorphisms in irinotecan-induced neutropenia in Asian cancer patients. Cancer Sci 98:1461–1467
9. Minami H, Sai K, Saeki M, Saito Y, Ozawa S, Suzuki K, Kaniwa N, Sawada J, Hamaguchi T, Yamamoto N, Shirao K, Yamada Y, Ohmatsu H, Kubota K, Yoshida T, Ohtsu A, Saijo N (2007) Irinotecan pharmacokinetics/pharmacodynamics and *UGT1A* genetic polymorphisms in Japanese: roles of *UGT1A1*6* and **28*. Pharmacogenet Genomics 17:497–504
10. Han JY, Lim HS, Shin ES, Yoo YK, Park YH, Lee JE, Jang IJ, Lee DH, Lee JS (2006) Comprehensive analysis of *UGT1A* polymorphisms predictive for pharmacokinetics and treatment outcome in patients with non-small-cell lung cancer treated with irinotecan and cisplatin. J Clin Oncol 24:2237–2244
11. Nozawa T, Minami H, Sugiura S, Tsuji A, Tamai I (2005) Role of organic anion transporter OATP1B1 (OATP-C) in hepatic uptake of irinotecan and its active metabolite, 7-ethyl-10-hydroxycamptothecin: in vitro evidence and effect of single nucleotide polymorphisms. Drug Metab Dispos 33:434–439
12. Xiang X, Jada SR, Li HH, Fan L, Tham LS, Wong CI, Lee SC, Lim R, Zhou QY, Goh BC, Tan EH, Chowbay B (2006) Pharmacogenetics of *SLCO1B1* gene and the impact of **1b* and **15* haplotypes on irinotecan disposition in Asian cancer patients. Pharmacogenet Genomics 16:683–691

13. Takane H, Miyata M, Burioka N, Kurai J, Fukuoka Y, Suyama H, Shigeoka Y, Otsubo K, Ieiri I, Shimizu E (2007) Severe toxicities after irinotecan-based chemotherapy in a patient with lung cancer: a homozygote for the *SLCO1B1*15* allele. Ther Drug Monit 29:666–668
14. Han JY, Lim HS, Shin ES, Yoo YK, Park YH, Lee JE, Kim HT, Lee JS (2008) Influence of the organic anion-transporting polypeptide 1B1 (*OATP1B1*) polymorphisms on irinotecan-pharmacokinetics and clinical outcome of patients with advanced non-small cell lung cancer. Lung Cancer 59:69–75
15. Rivory LP, Robert J (1995) Identification and kinetics of a β-glucuronide metabolite of SN-38 in human plasma after administration of the camptothecin derivative irinotecan. Cancer Chemother Pharmacol 36:176–179
16. Sparreboom A, de Bruijn P, de Jonge MJ, Loos WJ, Stoter G, Verweij J, Nooter K (1998) Liquid chromatographic determined of irinotecan and three major metabolites in human plasma, urine and feces. J Chromatogr B Biomed Sci Appl 721:225–235
17. Klein CE, Gupta E, Reid JM, Atherton PJ, Sloan JA, Pitot HC, Ratain MJ, Kastrissios H (2002) Population pharmacokinetic model for irinotecan and two of its metabolites, SN-38 and SN-38 glucuronide. Clin Pharmacol Ther 72:638–647
18. Marre F, Sanderink GJ, de Sousa G, Gaillard C, Martinet M, Rahmani R (1996) Hepatic biotransformation of docetaxel (Taxotere) in vitro: involvement of the CYP3A subfamily in humans. Cancer Res 56:1296–1302
19. Smith NF, Figg WD, Sparreboom A (2006) Pharmacogenetics of irinotecan metabolism and transport: an update. Toxicol In Vitro 20:163–175
20. Han JY, Lim HS, Park YH, Lee SY, Lee JS (2008) Integrated pharmacogenetic prediction of irinotecan pharmacokinetics and toxicity in patients with advanced non-small cell lung cancer. Lung Cancer. doi:10.1016/j.lungcan.2007.12.003
21. Nishizato NY, Ieiri I, Suzuki H, Kimura M, Kawabata K, Hirota T, Takane H, Irie S, Kusuhara H, Urasaki Y, Urae A, Higuchi S, Otsubo K, Sugiyama Y (2003) Polymorphisms of *OATP-C* (*SLC21A6*) and *OAT3* (*SLC22A8*) genes: consequences for pravastatin pharmacokinetics. Clin Pharmacol Ther 73:554–565