

RESEARCH ARTICLE

Prediction of Response to Irinotecan and Drug Toxicity Based on Pharmacogenomics Test: A Prospective Case Study in Advanced Colorectal Cancer

AH Emami¹, S Sadighi^{1*}, R Shirkoohi², MA Mohagheghi³

Abstract

Background: FOLFIRI regimen, which is composed of 5-FU, Leucovorin, and Irinotecan, is used in the first-line chemotherapy of metastatic colorectal cancer. Irinotecan life threatening toxicity is partly related to cytotoxic drug metabolite which is primarily inactivated by the UGT1A1 enzyme. The primary aim of the present research was to find the correlation between UGT1A1-genotype and clinical toxicity of irinotecan. **Methods:** In a prospective study from March 2011 to December 2013, all patients with metastatic colorectal cancer who had been referred to Medical Oncology Department of Iran Cancer Institute were genotyped for UGT1A1*28 before the first cycle of chemotherapy. All of the patients signed informed consent and trial approved by Ethics Committee of the Tehran University of Medical Sciences. Reduction of the standard dose of Irinotecan (180 mg/m² body surface area) was measured based on NCI toxicity criteria after the first cycle of chemotherapy. Patients with previous treatment with Oxaliplatin and fluorouracil (5-FU) in the adjuvant setting and adequate liver, kidney, and heart function were included in the trial. Both synchronous and metachronous metastatic disease were noticeable. **Results:** A total of 50 patients with median age of 52 years were included. Most (70%) of the patients had more than one site of metastases in peritoneum, liver, and/or lung. Thirty-one patients had UGT1A1*1 normal genotype, 13 were in heterozygote and 6 were in homozygote state of UGT1A1*28/*28. A clinically relevant increase in early toxicity was found in patients carrying the UGT1A1*28/*28 genotype with odds Ratio (OR) of 2.6 (95%CI 2.5-27.28). Similarly, there was a trend of lower overall survival in homozygote group with an HR (Hazardous Ratio) of 2.76 (95%CI .88-.61). No statistically significant relationship was found between UGT1A1 genotypes and response to therapy. **Conclusions:** UGT1A1 28*/28* is strongly associated with drug's life-threatening toxicity even in a moderate dose of Irinotecan. On the other hand, UGT1A1 genotype data was not helpful to differentiate response to treatment.

Keywords: Irinotecan- pharmacogenomic- toxicity- colon- cancer

Asian Pac J Cancer Prev, **18** (10), 2803-2807

Introduction

In practice of medicine, differences in response and toxicity of drugs have been reported and explored many times (Bandres et al., 2007). Interindividual differences in drug metabolism are caused by different mechanisms (Bandres et al., 2007). Finding specific metabolic pathways leading to severe toxicity could have a major role in the safe and selective treatment of cancer patients.

Irinotecan is used, either alone or in combination with 5-FU and folinic acid, for the treatment of metastatic colorectal cancer. The use of Irinotecan might be complicated with unpredictable severe toxicity (Evans and McLeod, 2003). In the past, many patients with Gilbert's syndrome showed severe toxicity of Irinotecan such as high-grade diarrhea and neutropenia, suggesting a genetic basis for the reaction (Salonga et al., 2000).

In fact, inter-individual differences in drug metabolism are an important cause of adverse drug reaction or lack of drug response (Sim et al., 2013; Tracy et al., 2016). Irinotecan active metabolite (SN-38) is primarily inactivated in the liver by the bilirubin metabolizing enzyme UDP-glucuronosyltransferase 1A1 (UGT1A1). There are reports of polymorphism of UGT1A1 (), which results in severe toxicity in rapidly dividing cells, leading to myelosuppression and delayed type diarrhea (Ratain et al., 2002; Bandres et al., 2007).

We prospectively genotyped 50 Iranian Irinotecan treated patients, to find the correlation between UGT1A1-genotype and clinical toxicity of irinotecan. Secondary end points were analyzing patients' survival and response rate based on polymorphism of the enzyme.

¹Hematology-Medical Oncology Department, ³Surgery-Oncology Department, Cancer Research Center, ²Group of Genetics, Cancer Biology Research Center, Cancer Institute of Iran, Tehran University Medical Sciences, Tehran, Iran. *For Correspondence: s.sadighi@hotmail.com

Material and Methods

From March 2011 to December 2013, fifty patients with metastatic colorectal cancer who were treated primarily with the study protocol were registered for the present study at the Medical Oncology Department of Iran Cancer Institute. The study was approved by the ethics committee of Tehran University of Medical Sciences (TUMS). Before beginning the chemotherapy, 2 ml whole EDTA blood was taken from patients and shipped to Central Lab (Parto- Lab) within a maximum of two hours, keeping the sample at 4 °C. QIA amp DNA Blood Mini-kit was used to extract DNA from samples and Nano-Drop instrument was utilized for quality control of the extracted DNA. Genotyping was performed by PCR and DNA sequencing mentioned precisely in following references (Innocenti et al., 2004; Marsh and Hoskins, 2010; Suzuki et al., 2012).

Inclusion criteria required signed informed consent, pathology report of colorectal adenocarcinoma, measurable metastatic disease based on RESIST 1.1 criteria, normal liver, heart, kidney and hematologic laboratory parameters, and good performance status (0-2 WHO scores). Previous chemotherapy in the adjuvant setting was allowed.

Exclusion criteria included having a plan of surgery as metastasectomy, baseline bilirubin more than 2mg/dl, liver enzymes more than 3 times the upper limit of the normal range, age 75 or more and inability to do personal care (poor performance status). None of the patients had taken chemotherapy in the metastatic setting before.

Technical consideration of genotyping

Irinotecan metabolizing risk categories groups were defined as low-risk: common allele UGT1A1: (1*1*), moderate risk: heterozygote for polymorphism UGT1A1: (1*28*) and high risk: homozygote for polymorphism UGT1A1: (28*28*).

Planned chemotherapy protocol (FOLFIRI) was identified as Irinotecan (Pfizer) 180mg/m² day1, Leucovorin 400mg/m² and 5FU 400mg/m² bolus day 1, then 5FU 2400mg/m² Iv 46h. The protocol was repeated every two weeks, for 12 cycles.

The researchers of the present study used NCI v3 drug reaction and QLQ-C30 questionnaires to check toxicity and patients' quality of life retrospectively. A few years ago, QLQ-C30 questionnaire was translated into Farsi and validated for Iranian clinical trials (Montazeri et al., 1999).

The standard moderate dose of Irinotecan was used in the present study. However, the procedure followed by decreasing 20% of Irinotecan and 5-FU dose in the subsequent cycle based on the observation of grade III or IV drug adverse reactions. Investigators received genomic test result after the second cycle of chemotherapy. Therefore, the test result had no role on the first dose modifications of Irinotecan. All of the patients received prophylactic G-CSF (Neupogen Roche) support.

In the case of late diarrhea, oral Loperamide and Ciprofloxacin were administered. Patients with febrile neutropenia were hospitalized with IV fluid and antibiotics therapy.

We used SPSS v 19 for analyses of data. Odds ratios

(OR) were calculated with logistic regression. Hazard ratios (HR) were analyzed using Cox regression.

Results

A total of 50 patients were registered in the present study and all of them followed to death or end of 2015. The median age of the patients was 52 years (+/-12.4). Most of the patients (70%) had more than one metastases site in peritoneum, liver, and/or lung and 13 had permanent colostomies. Table 1 shows demographic characteristics of the patients.

The median of overall survival was 21 months (14-27). Toxicity was most frequently associated with leukopenia and diarrhea followed by infection, anemia, and fatigue. Only 2 patients showed no drug reaction. There were 25 early discontinuations of chemotherapy protocol. 9 cases

Table 1. Demographic Characteristics of the Patients

Characteristic	FOLFIRI* (n=50)	
Age (years)		
Mean (s.d.)	52.2	(12.4)
Median (range)	53	(51)
WHO P.S., n (%)		
First cycle		
0	6	(12.2)
1	17	(34.7)
2	23	(46.9)
3	3	(6.1)
Second cycle		
0	2	(4.1)
1	14	(28.6)
2	17	(34.7)
3	16	(32.6)
Colectomy, n (%)		
Yes/No	13/37	(26/74)
Location of Metastasis, n (%)		
Liver	7	(14)
Lymph node	21	(42)
Lung	4	(8)
Perit	6	(12)
Other	12	(24)
No. of organs involved, n (%)		
1	14	(29.8)
2	22	(46.8)
3+	11	(23.4)
Haemoglobin, mean (range)	11.7	(8.7)
Low haemoglobin, n (%) (<12)	24	(48)
P-bilirubin, mean (range)		
First cycle	2	(11)
Second cycle	1.1	(9.6)
Overall Survival, median (range)	29	(17)

*FOLFIRI, Combination chemotherapy included; 5-FU, Leucovorin, Irinotecan

Table 2. Relationship between Genotype of Irinotecan and Clinical Toxicity of FOLFIRI Chemotherapy

Outcome	All	Toxicity (%)	No toxicity (%)	OR	(95% CI)	P-value (exact)
Genotype						
Normal	31	26 (83.9)	5 (16.1)	1		(0.004)
Heterozygote	13	9 (69.2)	4 (30.8)	2.3	(0.5-10.5)	
Homozygote	6	1 (16.7)	4 (30.8)	26	(2.5-272.8)	

Table 3. Patients Performance Status Changes after Chemotherapy

Genotype	All	Normal (%)	Heterozygote (%)	Homozygote (%)	Kendall's Tau	P-value (exact)
WHO P.S						
1 st Cycle						
0	6	4 (66.7)	2 (33.3)	0 (0)	-0.0227	(0.785)
1	17	10 (58.8)	3 (17.6)	4 (23.5)		
2	23	14 (60.9)	7 (30.4)	2 (8.7)		
3	3	2 (66.7)	1 (33.3)	0 (0)		
2 nd Cycle						
0	2	2 (100)	0 (0)	0 (0)	0.1666	(0.946)
1	14	9 (64.3)	4 (23.5)	1 (7.1)		
2	17	11 (64.7)	4 (31.2)	2 (11.8)		
3	16	8 (50)	5 (26.5)	3 (18.7)		

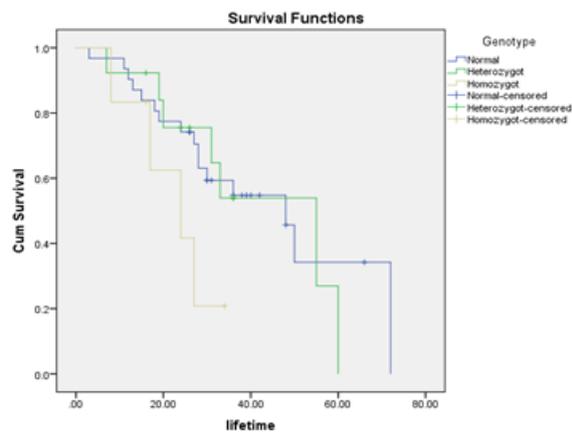


Figure 1. Effect of Irinotecan Pharmacogenomics on Survival of the Patients in Months

were associated with severe chemotherapy toxicity and 16 with progressing disease. Table 2 shows drug toxicity based on NCI criteria.

Table 3 presents drug genotyping results. Thirty-one patients had UGT1A1*1, thirteen were heterozygous, and 6 were homozygous for UGT1A1*28/*28. A clinically relevant increase in early toxicity was observed in patients carrying the UGT1A1*28/*28 genotype with an OR of 2.6(95%CI 2.5-272.8). Similarly, there was a trend of lower overall survival in homozygote group with an HR of 2.76. A statistically significant relationship was not found between the genotype and response to therapy.

Although the sample size was limited, UGT1A1 28*/*28* showed 2.7 times more association with the life-threatening toxicity of Irinotecan compared to normal variant. On the other hand, there was no significant difference between normal genotype and heterozygote

state in relation to toxicity or survival (Figure 1).

EORTC QLQ-C30 questionnaire results would be reported in another paper.

Discussion

In the present study, the relationship between Irinotecan efficacy and toxicity and pharmacogenomic of the drug metabolizing enzyme UGT1A1 was evaluated. It is obvious that there are individual differences with regard to toxicity and response to the anti-cancer drugs irrespective of age, sex, body surface area, drug interactions, and tumor stage or organ functions. In fact, disease response may be related to interindividual differences in surface proteins or enzymes interaction in drug metabolism. In this sense, one of the aspects of the personalized medicine is considering pharmacogenomic of drugs (Swen et al., 2011).

Although there are reports of fatal toxicity of Irinotecan based on high doses (more than 250mg/m²) (Hoskins et al., 2007; Swen et al., 2011), the finding of the present study revealed significant toxicity in colorectal cancer patients treated with moderate doses(180mg/m²) of Irinotecan. Unpredictable severe toxicity of Irinotecan is also addressed in other studies (Hoskins et al., 2007; Tsunedomi et al., 2014). There are reports on risk of severe toxicity in patients treated with medium doses of irinotecan in relation to homozygosity for UGT1A1*28. For example, in the randomized controlled phase III trial Nordic VI, which, compared the effects of irinotecan either with bolus 5-FU or bolus/infused 5-FU, the prescribed dose of irinotecan was 180/m² (Glimelius et al., 2011). They found 46% grade III or IV toxicity excluding alopecia. The most common toxicities were diarrhea and neutropenia. Nordic investigators found an increased

risk of early Irinotecan-induced toxicity in patients homozygous for the UGT1A1*28 variant (Glimelius et al., 2011).

Only 12% of our cases showed homozygote state, but 2 of our patients died because of unpredictable severe early toxicity of chemotherapy. It could be a big challenge; on one hand, in 2005, FDA approved the inclusion of UGT1A1 genotype-associated risk of toxicity on the Irinotecan package insert (<http://www.fda.gov/medwatch/SAFETY2005>); on the other hand, there are concerns about globally checking DNA sequences of UGT1A1 genes due to cost and time consumption of the test, limited available sites for detection of common UGT1A1 alleles and last but not the least, problems related to quality control of the test (emedicine.medscape.com/article/1790367-overview).

The present study indicated no statistical differences in toxicity between normal and heterozygote state of UGT1A1 genotype in metabolizing medium dose of Irinotecan in FOLFIRI regimen. Another study reported 50% and 12.5% grade III or IV toxicity in homozygote and heterozygote group of patients based on UGT1A1 genotyping with in response to using single agent Irinotecan in 350mg/m²dose (Tucano, Sugiyama, 2017).

The present study has many limitations. Firstly, the sample size of the research was too small. Secondly, because of technical and financial problems in checking mutations of Dihydropyrimidine-dehydrogenase (DHPD) and Thymidylate Synthase (TS) by PCR test, the probable role of polymorphism of 5-FU metabolism on severe toxicity of chemotherapy protocol cannot be ignored (Lecomte et al., 2004; Keiser, 2008; Amstutz et al., 2009; Loganayagam et al., 2010).

Thirdly, in this study only UGT1A1 genotype was checked, but not other glucuronosylation enzymes included in metabolism of Irinotecan addressed which were addressed in other studies (Glimelius et al., 2011; Swen et al., 2011). Therefore, this investigation should be considered as an exploratory research work.

Fourthly, Investigators used G-CSF generously after any cycle of chemotherapy to prevent febrile neutropenia. It causes low number of neutropenia between cycles of chemotherapy. However, two patients with a severe metabolic defect of Irinotecan died of diarrhea and sepsis that shows the importance and deadly problem of patients with homozygote state who are receiving a standard dose of the drug. They probably need lower doses of the drug from the start of treatment.

In conclusion, every oncologist likes to save his or her patients to receive the best response without obtaining too much toxicity. Genotyping of main metabolizing enzymes of Irinotecan is an action to avoid of unpredictable and life-threatening toxicity confronting homozygote patient, while others can receive the higher doses of drug safely.

It is important to pay attention to the baseline bilirubin level in serum of the patient, and family history of Gilbert disease. Patients with baseline serum total bilirubin levels of 1.0 mg/dL or more have a greater likelihood of first-cycle grade 3 or 4 toxicity than those with less bilirubin level. Patients with deficient glucuronosylation

of bilirubin, such as those with Gilbert's syndrome, may be at a greater risk of myelosuppression when receiving therapy with Irinotecan Hydrochloride. It should be noted that the problem is very important and even life threatening in case of using medium to high doses of Irinotecan (180-350mg/m²).

Funding Statement

This study was supported by Tehran University of Medical Sciences, Tehran, Iran (grant number 11613).

Acknowledgments

The authors wish to thank all colleagues who helped to carry out this study. We especially are thankful of Dr. Mehran Ghaderi for comments on the first draft of the manuscript and Dr. Kazem Zendejdel and Ali Rafeei for their help on analyzing of data.

References

- Amstutz U, Farese S, Aebi S, et al (2009). Dihydropyrimidine dehydrogenase gene variation and severe 5-fluorouracil toxicity: a haplotype assessment. *Pharmacogenomics J*, **10**, 931-44.
- Bandres E, Zarate R, Ramirez N, et al (2007). Pharmacogenomics in colorectal cancer: the first step for individualized-therapy. *World J Gastroenterol*, **13**, 5888-901.
- Evans WE, McLeod HL (2003). Pharmacogenomics--drug disposition, drug targets, and side effects. *N Engl J Med*, **348**, 538-49.
- Glimelius B, Garmo H, Berglund A, et al (2011). Prediction of irinotecan and 5-fluorouracil toxicity and response in patients with advanced colorectal cancer. *Pharmacogenomics J*, **11**, 61-71.
- Hoskins JM, Goldberg RM, Qu P, et al (2007). UGT1A1*28 genotype and irinotecan-induced neutropenia: dose matters. *J Natl Cancer Inst*, **99**, 1290-5.
- Innocenti F, Undevia SD, Iyer L, et al (2004). Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. *J Clin Oncol*, **22**, 1382-8.
- Keiser W (2008). The role of pharmacogenetics in the management of fluorouracil-based toxicity. *Commun Oncol*, **5**, 1-8.
- Lecomte T, Ferraz JM, Zinzindohoue F, et al (2004). Thymidylate synthase gene polymorphism predicts toxicity in colorectal cancer patients receiving 5-fluorouracil-based chemotherapy. *Clin Cancer Res*, **10**, 5880-8.
- Loganayagam A, Arenas-Hernandez M, Fairbanks L, et al (2010). The contribution of deleterious DPYD gene sequence variants to fluoropyrimidine toxicity in British cancer patients. *Cancer Chemother Pharmacol*, **65**, 403-6.
- Marsh S, Hoskins JM (2010). Irinotecan pharmacogenomics. *Pharmacogenomics*, **11**, 1003-10.
- Montazeri A, Harirchi I, Vahdani M, et al (1999). The European organization for research and treatment of cancer quality of life questionnaire (EORTC QLQ-C30): translation and validation study of the Iranian version. *Supp Care Cancer*, **7**, 400-6.
- Ratain LI, Das S, Janisch L, et al (2002). UGT1A1[ast]28 polymorphism as a determinant of irinotecan disposition and toxicity. *Pharmacogenomics J*, **2**, 43-7.
- Salonga D, Danenberg KD, Johnson M, et al (2000). Colorectal tumors responding to 5-fluorouracil have low gene

- expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase, and thymidine phosphorylase. *Clin Cancer Res*, **6**, 1322-7.
- Sim SC, Kacevska M, Ingelman-Sundberg M (2013). Pharmacogenomics of drug-metabolizing enzymes: a recent update on clinical implications and endogenous effects. *Pharmacogenomics J*, **13**, 1-11.
- Suzuki S, Komori M, Hirai M, et al (2012). Development of a novel, fully-automated genotyping system: principle and applications. *Sensors (Basel)*, **12**, 16614-27.
- Swen JJ, Nijenhuis M, de Boer A, et al (2011). Pharmacogenetics: from bench to byte--an update of guidelines. *Clin Pharmacol Ther*, **89**, 662-73.
- Tacano M, Sugiyama T (2017). UGT1A1 polymorphism in cancer: impact on irinotecan treatment. *Pharmacogenomics Pres Med*, **10**, 61-8
- Tracy TS, Chaudhry AS, Prasad B, et al (2016). Interindividual variability in cytochrome P450-mediated drug metabolism. *Drug Metab Dispos*, **44**, 343-51.
- Tsunedomi R, Hazama S, Fujita Y, et al (2014). A novel system for predicting the toxicity of irinotecan based on statistical pattern recognition with UGT1A genotypes. *Int J Oncol*, **45**, 1381-90.