Pharmacological Research 136 (2018) 133–139

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Clinical utility of *ABCB1* genotyping for preventing toxicity in treatment with irinotecan

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ARTICLE INFO

Keywords:
Adverse reactions
Pharmacogenetics
SNP
Cancer
UGT1A1
ABC transporters

ABSTRACT

Preventing severe irinotecan-induced adverse reactions would allow us to offer better treatment and improve patients' quality of life. Transporters, metabolizing enzymes, and genes involved in the folate pathway have been associated with irinotecan-induced toxicity. We analyzed 12 polymorphisms in *UGT1A1*, *ABCB1*, *ABCG2*, *ABCC4*, *ABCC5,* and *MTHFR* in 158 patients with metastatic colorectal cancer treated with irinotecan and studied the association with grade > 2 adverse reactions (CTCAE). Among the most frequent ADRs, the SNPs rs1128503, rs2032582, and rs1045642 in *ABCB1* and rs1801133 in *MTHFR* were associated with hematological toxicity and overall toxicity. The SNP rs11568678 in *ABCC4* was also associated with overall toxicity. After correction of *P* values using a false discovery rate, only *ABCB1* variants remained statistically significant. Haplotype analysis in *ABCB1* showed an 11.3-fold and 4.6-fold increased risk of hematological toxicity (95% CI, 1.459–88.622) and overall toxicity (95% CI, 2.283–9.386), respectively. Consequently, genotyping of the three SNPs in *ABCB1* can predict overall toxicity and hematological toxicity with a diagnostic odds ratio of 4.40 and 9.94, respectively. Genotyping of *ABCB1* variants can help to prevent severe adverse reactions to irinotecan-based treatments in colorectal cancer.

1. Introduction

Irinotecan is a cytotoxic drug used for the treatment of solid tumors, mainly advanced colorectal cancer (CRC) [1]. Although it can be administered in monotherapy, it is usually co-administered with a fluoropyrimidine, either 5-fluorouracil (5-FU) or capecitabine, as part of the chemotherapeutic combination regimens FOLFIRI and XELIRI, respectively. In metastatic disease, these regimens can also include a monoclonal antibody such as bevacizumab or cetuximab. The effectiveness of these combined treatments has increased survival rates in the last 20 years and aggravated associated severe adverse reactions [2]. Irinotecan-induced toxicity is a limiting factor in the treatment of solid tumors. As with other cytotoxic agents, irinotecan is associated with severe adverse reactions, especially neutropenia and diarrhea [3]. Toxicity is dose-dependent, and the highest rates are found in patients receiving irinotecan at doses greater than 180 mg/m^2 or in combination with a fluoropyrimidine [4,5]. The profile and severity of toxicity increase when irinotecan is combined with fluoropyrimidines. Many treatment schedules need doses to be decreased or administration to be delayed because of toxicity, thus limiting the success of chemotherapy. Knowledge of predictors could help us to avoid irinotecan-induced toxicity and improve treatment and quality of life.

Irinotecan acts by inhibiting topoisomerase I, an enzyme participating in the correction of transcription errors and in cell proliferation [6]. The antitumoral activity of irinotecan is mainly due to SN-38, the product of carboxylesterase-mediated hydrolysis in the liver. Subsequently, approximately 70% of SN-38 is glucuronidated by UGT1A1 to become SN-38 G, which has 1/100 of the antitumor activity and is virtually inactive [7].

The number of repeats of a dinucleotide (TA) in a TATA box is

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https://doi.org/10.1016/j.phrs.2018.08.026
Received 30 May 2018; Received in revised form 30 July 2018; Accepted 31 August 2018
Available online 11 September 2018

related to mRNA expression of *UGT1A1*, efficacy, and toxicity and defines allele *28 [8,9]. *UGT1A1**28 is currently the only pharmacogenetic biomarker with a recognized role in preventing severe irinotecan-induced toxicity. In individuals who are homozygous for *UGT1A1**28, the Royal Dutch Association for the Advancement of Pharmacy-Pharmacogenetics Working Group (DWCP) recommends a 30% reduction for doses higher than 250 mg/m^2, while a French Consortium recommends a 25–30% reduction for doses between 180 and 230 mg/m^2 and considers doses higher 230 mg/m^2 to be contraindicated [5,10]. Given that the most frequently administered dose of irinotecan is 180 mg/m^2 and that the recommendations in these guidelines are contradictory, it is necessary to increase our knowledge of irinotecan, especially in patients undergoing treatment.

In addition, since *UGT1A1* is not the only gene involved in the pharmacokinetics and pharmacodynamics of irinotecan, other enzymes and transporters may have a relevant role in the development of toxicity. Therefore, it is necessary to identify more genetic variants to help explain the toxicity observed. For instance, ATP-binding cassette (ABC) transporters play a key role in transporting irinotecan and SN-38 out of the cell. DNA variants in ABC transporters are good candidates for altering drug availability in cells and can contribute to adverse reactions [11,12]. Thus, SNPs in *ABCB1* and in other ABC transporters have also been associated with irinotecan-induced toxicity, and findings have been controversial [12–17].

ABCC4 and *ABCC5* have been associated with toxicity in renal tubular cells, although little information has been reported about irinotecan-induced toxicity [12]. Furthermore, given that irinotecan increases the likelihood of adverse reactions induced by the fluoropyrimidines that are often co-administered with irinotecan, DNA variants involved in the pharmacodynamics or pharmacokinetics of fluoropyrimidines associated with fluoropyrimidine-induced toxicity may be related to administration of irinotecan. In this sense, controversial results have been reported for *MTHFR* variants in fluoropyrimidine-based chemotherapy, although these variants have also been associated with irinotecan-induced toxicity [18].

Increasing our knowledge of irinotecan-induced toxicity and potentially associated biomarkers could help to establish a predictive test to prevent severe adverse reactions and to select optimal treatment.

In this study, we analyzed 12 SNPs in *UGT1A1*, in three ABC efflux transporter genes (*ABCB1*, *ABCC4,* and *ABCC5*), and in *MTHFR* to identify variants associated with irinotecan-induced toxicity and to establish a predictive test for toxicity.

2. Material and methods

2.1. Patients

The present study was an observational, ambispective case-control analysis. Patients were recruited between 2007 and 2015 as part of a wider study population. A small part of the recruited patients participated in previous studies [19–22]. The inclusion criteria were diagnosis of metastatic CRC treated with an irinotecan-containing regimen in any line of treatment, performance status ≤ 2, and age ≥ 18 years. Samples from patients were provided by the medical oncology departments of four hospitals (Hospital General Universitario Gregorio Marañón, Hospital Universitario La Paz, Hospital Universitario Doce de Octubre, and Hospital Universitario Ramón y Cajal). Samples were collected in tubes containing EDTA and frozen immediately on reception at –80 °C. The demographic and clinical data collected included age, sex, type of cancer (colon or rectal), and concomitant drugs. The adverse drug reactions (ADRs) recorded included those most frequently associated with irinotecan: nausea and vomiting, diarrhea, mucositis and stomatitis, anorexia, skin toxicity, hematological toxicity, hepatic toxicity (elevated bilirubin or transaminases), and asthenia. Toxicity was graded according to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE) of the National Cancer Institute by the attending physician, during the course of standard medical care. Adverse events were collected form clinical records and available laboratory results during the time of irinotecan-based chemotherapy. Overall toxicity, defined as the presence of any ADR higher than grade 2, was also analyzed. The study was conducted in accordance with the Declaration of Helsinki and Spanish laws and was approved by the ethics committees of the hospitals involved. Informed consent was obtained from all patients.

2.2. DNA isolation and genotyping

Genomic DNA was isolated from 200 µl of whole blood using the High Pure PCR template preparation kit (Roche Applied Sciences, Penzberg, Germany). The DNA concentration was measured using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA). The polymorphisms rs1801133 and rs1801131 in *MTHFR*, rs1128503, rs2032582, and rs1045642 in *ABCB1*, rs4148551 and rs37421551 in ABCC4, rs3805114 in *ABCC5,* and rs10929302 in *UGT1A1* were genotyped using SNaPshot, as previously described [20,23]. The SNPs rs11568658 in *ABCC4* and rs2231142 in *ABCG2* were genotyped using TaqMan probes in a StepOnePlus Real-Time PCR System (Life Technologies, Carlsbad, California, USA). Allele discrimination was performed using StepOne software v2.3. Allele *28 in *UGT1A1* (rs8175347) was genotyped using PCR product fragment length and electrophoresis [24]. Hardy-Weinberg equilibrium was analyzed to detect deviations in genotype frequency [25].

2.3. Statistical analysis

All data were analyzed using the Statistical Package for the Social Sciences v.15 (SPSS, Inc). A linear-by-linear association chi-square test was used to investigate the univariate associations between polymorphisms and capecitabine-related ADRs. *P* values were also adjusted using the Benjamini and Hochberg false discovery rate (FDR) with R v3.3.3. The *P* value, odds ratio (OR), and 95% confidence interval (CI) were analyzed using multivariate logistic regression for *ABCB1* haplotypes. The covariates analyzed using multivariate logistic regression were sex, hospital, and treatment line. A P value < 0.05 was considered statistically significant. The positive predictive value (PPV), negative predictive value (NPV), sensitivity, specificity, accuracy, and diagnostic odds ratio were calculated as described elsewhere [26].

3. Results

3.1. Patients

The study population comprised 158 patients with metastatic CRC who were treated with irinotecan. The baseline characteristics of the study patients are shown in Table 1. The study population comprised mainly men (58.2%) diagnosed with colon cancer (66.4%), most of whom were recruited in two of the participating hospitals. Most were treated with 5-FU as a concomitant fluoropyrimidine (65.2%), and the main antibody therapy was bevacizumab (41.8%). At least one severe ADR was recorded in 39.9% of patients. The most frequent ADRs were hematological toxicity (14.5%), asthenia (7.6%), and diarrhea (6.3%).

3.2. Association between DNA variants and toxicity

All the observed genotype frequencies were consistent with the Hardy-Weinberg equilibrium model. Associations between DNA variants and severe adverse reactions were analyzed using a codominant model (Supplemental Table 1). A preliminary analysis showed that the SNPs rs1128503, rs2032582, and rs1045642 in *ABCB1* and rs1801133 in *MTHFR* were associated with overall toxicity and hematological toxicity; in addition, rs11568658 in *ABCC4* was associated with overall toxicity; *UGT1A1* rs10929302 was associated with hyperbilirubinemia; rs2032582 in *ABCB1* was associated with severe nausea/vomiting and