Abstract. Background: Dihydropyrimidine dehydrogenase (DPD) is the rate-limiting enzyme of the degradation of pyrimidine base, and plays a pivotal role in the pharmacogenetic syndrome of 5-fluorouracil (5-FU). Deficiency of DPD activity leads to severe toxicities, even death, following administration of 5-FU. Several studies have demonstrated that molecular defects of the dihydropyrimidinedehydrogenase gene (DPYD) lead to the deficiency of DPD activityand cause this pharmacogenetic syndrome. We present the analysis of DPYD genotyping in untreated Caucasian patients (control group) and Caucasian patients with 5-FU/CAP-related grade 3/4 toxicities (toxicity group) who underwent a capcitabine TheraGuide 5-FU testing. Patients and Methods: Full sequencing of DPYD was performed in the Myriad Genetic Laboratories, Inc. as part of TheraGuide 5-FU test. Results: Among 227 patients from the toxicity group, 27 (12%) had deleterious mutations in DPYD: twelve (5%) had IVS14 +1 G>A, eleven (5%) had D949V and four (2%) had other mutations. Only 7/192 (4%) patients from the control group had DPYD genotype abnormalities: two (1%) had IVS14 +1 G>A, four (2%) had D949V and one (1%) had other mutation. Genotype abnormalities were observed more frequently in the toxicity group (p=0.001). Among 65 patients with toxicities due to capcitabine, nine (14%) had mutated DPYD, which was more frequent than in the control group (p=0.006). Conclusion: Mutated DPYD is frequently observed in Caucasian patients who experience toxicities while receiving 5-FU/capcitabine. Screening of patients for DPYD mutations prior to administration of 5-FU/capcitabine using new pharmacogenetic testing methods, may help for identify those patients who are at greatest risk for adverse effects, allowing a more individualized approach to their chemotherapy management.

As many as one in three patients receiving 5-FU/capcitabine experience dose-limiting toxicities including diarrhea, hand-foot syndrome (HFS), mucositis and myelosupression (1). Deficiency of DPD, a rate-limiting enzyme in 5-FU catabolism, can lead to life-threatening complications (2). DPD deficiency was found to be related to >40 sequence variations in DPYD (3). Known deleterious mutations explain only a limited proportion of adverse events due to 5-FU. Full sequence analysis of the DPYD gene increases sensitivity by 20% vs. simple identification of two most common deleterious mutations (IVS14 +1 G>A, D949V) (4). Pre-treatment detection could prevent serious, potentially lethal side effects (5).

The current report presents results of an analysis of DPYD genotyping for untreated Caucasian patients (control group) and Caucasian patients with 5-FU/capcitabine-related grade 3/4 toxicities (toxicity group) who underwent TheraGuide 5-FU testing.

Patients and Methods

Full sequencing of DPYD was performed by the Myriad Genetic Laboratories, Inc. as part of a TheraGuide 5-FU test (Myriad Genetic Laboratories, Inc., Salt Lake City, UT, USA) (6). DNA was extracted and purified from white blood cells. Genetic variants in patient’s DPYD were detected by comparison with a consensus wild-type DPYD sequence. Analysis consisted of PCR and DNA sequencing of all 23 coding exons and approximately 690 adjacent intronic base pairs of the DPYD gene. Certain variations in the DPYD genes were associated with up to 60% risk of dose-limiting toxicity from 5-FU/capcitabine-based therapies. Patients carrying these variations should be managed differently, taking into account their high-risk status for adverse reactions. Test results for TheraGuide 5-FU™ are graded as shown in Table I. A patient is considered “High Risk” if regardless of the thymidinesynthase gene (TYMS) genotype, DPYD includes three known mutations (IVS14...
plus variants with significant evidence indicating that they adversely affect protein production or function. TYMS 2R/2R is considered to be of moderate risk but DPYD mutations are considered as high-risk for toxicity of 5-FU.

Results

A total of 1407 specimens were tested. Most of the patients had colon (n=871, 62%) or breast (n=184, 13%) cancers. There were 764 Caucasians and 229 non-Caucasian patients identified. A further 414 patients had multiple or unspecified ancestries. Age distribution among all tested patients as well as in Caucasian and non-Caucasian sub-groups is presented in Table II.

Deleterious mutations were identified in 95 patients and suspected deleterious mutations were identified in an additional seven patients (Table III).

Among 227 patients from the toxicity group, twenty seven (12%) had deleterious mutations in DPYD: twelve (5%) had IVS14 +1 G>A, eleven (5%) had D949V and four (2%) had other mutations. Only 7192 (4%) patients from the control group had DPYD genotype abnormalities: two (1%) had IVS14 +1 G>A, four (2%) had D949V and one (1%) had other mutation. Genotype abnormalities were seen more frequently in the toxicity group (p=0.001). Among 49 patients with hand-foot syndrome 9/49 (18%) had mutated DPYD. A total of 9/46 (20%) patients with myelosupression were found to have a deleterious mutation. Mutations were more frequent in both sub-groups when compared to the control group: p=0.001 and 0.0007 respectively. Among 65 patients with toxicities due to capecitabine, a total of nine (14%) had mutated DPYD which was more frequent than in the control group (p=0.006).

Discussion

Mutated DPYD is frequently observed in Caucasian patients who experience toxicities while receiving 5-FU/capecitabine. Screening of patients for DPYD mutations prior to administration of 5-FU/capecitabine using new pharmacogenetic testing methods may help to identify patients who are at greatest risk for adverse effects, allowing for a more indivi-dualized approach to their chemotherapy management (7).

A polymorphic abnormality of DPYD, a known pharmacogenetic syndrome associated with 5-FU toxicity, has been detected in 3% to 5% of the population (8). This abnormality and DPD deficiency can be identified by genetic analysis and by determination of DPD levels in the peripheral blood mononuclear cells, respectively (9). DPYD is the gene that codes for DPD. At least 34 types of DPYD variants have been reported to date (10-24). The mutation IVS14 + 1 G>A, DPYD*2A is the most common mutation associated with
clinical DPD deficiency. This mutation was detected in 24-28% of all patients suffering from severe 5-FU toxicity (4). Screening for this mutation may identify up to 60% of individuals with absolute DPD deficiency, who are at greatest risk of toxicity. Down-regulation by methylation of the DPD promoter region has been identified as one of the more important regulatory mechanisms of DPD enzymatic activity. Although resistance to 5-FU depends on many factors, tumoral DPD activity is a determining factor in predicting responsiveness to 5-FU.

While there are no formal recommendations that specifically address testing for DPD deficiency, the FDA does have a statement on its Web site that reads: “The U.S. Food and Drug Administration reported in its March 2003 Safety Labeling Changes Approved by FDA Center for Drug Evaluation and Research (CDER) that Xeloda® (capecitabine) is contraindicated in patients who have a known dihydropyrimidine dehydrogenase (DPD) deficiency” (25). This warning appears in the Xeloda® package insert. Additionally, the manufacturers of 5-FU have issued warnings and/or contraindications for patients with DPD enzyme deficiency. We have published our previous experience on this issue (26).

Patients with certain DPDY variants are at high risk of toxicity and may be indicated for effective alternate chemotherapeutic agents (27). Patients at high risk of toxicity may also be indicated for enhanced patient monitoring strategies to limit the incidence and severity of adverse reactions. There may be other, less common factors that affect risk for toxicity.

Conflicts of Interest

Dr. Saif has no conflicts of interest to disclose.

References


Received March 21, 2013
Revised April 8, 2013
Accepted April 9, 2013