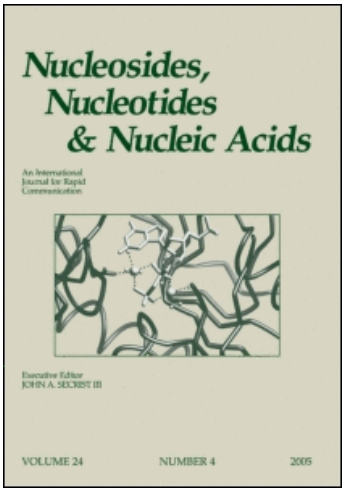

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ALTERED DIHYDROPYRIMIDINE DEHYDROGENASE ACTIVITY ASSOCIATED WITH MILD TOXICITY IN PATIENTS TREATED WITH 5-FLUOROURACIL CONTAINING CHEMOTHERAPY

André B. P. van Kuilenburg,[1] Heinz-Josef Klumpen,[2] Anneke M. Westermann,[2] Lida Zoetekouw,[1] Piet J. M. Bakker,[2] Henk-Jan Guchelaar,[3] and Dick J. Richel[2]

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□ *Dihydropyrimidine dehydrogenase (DPD) plays a pivotal role in the metabolism of 5-fluorouracil (5FU). In patients treated with capecitabine or 5FU combined with other chemotherapeutic drugs, DPD activity in peripheral blood mononuclear cells was increased in patients experiencing grade I/II neutropenia. In contrast, decreased DPD activity proved to be associated with grade I/II dermatological toxicity, including hand-foot syndrome. Thus, patients with a low-normal or high-normal DPD activity proved to be at risk of developing mild toxicity upon treatment with 5FU-based chemotherapy, demonstrating the important role of DPD in the etiology of toxicity associated with 5FU and the catabolites of 5FU.*

Keywords Dihydropyrimidine dehydrogenase; FBAL; 5-fluorouracil; fluoro-5;6-dihydrouracil; toxicity

INTRODUCTION

5-Fluorouracil (5FU) and the oral prodrug capecitabine (Xeloda) are two of the most frequently prescribed chemotherapeutic drugs for the curative and palliative treatment of patients with cancers of the gastrointestinal tract, breast and head and neck.[1,2] Several studies have shown a clinical benefit of adding irinotecan or oxaliplatin to the treatment schedule with 5FU or capecitabine.[1,3,4] Nevertheless, the toxicity associated with these treatment schedules can be profound.[1]

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In this respect, it has been shown that dihydropyrimidine dehydrogenase (DPD) plays a pivotal role in the metabolism of 5FU.[5,6] More then 80% of the administered 5FU is catabolised by DPD and patients with a complete or partial DPD deficiency have a strongly reduced capacity to degrade 5FU.[7,8] Owing to the fact that 5FU has a relatively narrow therapeutic index, those patients with a complete or partial DPD deficiency have an increased likelihood of suffering from severe and sometimes even lethal drug-induced toxicity.[9–11] In this study, we have investigated the prognostic significance of the DPD activity in peripheral blood mononuclear (PBM) cells of cancer patients treated with 5FU-based chemotherapy, with respect to toxicity.

METHODS

Patients

The patient group consisted of 27 cancer patients who were treated in the Academic Medical Center with capecitabine or a 5FU-containing regimen. None of these patients received chemotherapy at the time of blood sampling for determination of the DPD activity. All samples were obtained between 10 a.m. and 12 a.m. The toxicity experienced by the patients upon subsequent treatment with 5FU-based chemotherapy was graded in accordance with the Common Toxicity Criteria.

Determination of the DPD Activity

PBM cells were isolated from 15 ml EDTA-anticoagulated blood by centrifugation over lymphoprep and the cells from the interface were collected and treated with ice-cold NH_4Cl to lyse the contaminating erythrocytes, as described before.[12] The activity of DPD was determined using radiolabeled thymine followed by separation of radiolabeled thymine from radiolabeled dihydrothymine using reversed-phase HPLC with online detection of the radioactivity.[12] Protein concentrations were determined with a copper-reduction method using bicinchoninic acid, essentially as described by Smith et al.[13]

RESULTS

Patients Characteristics and Clinical Presentation

The characteristics of the patients are summarized in Table 1. The majority of the patients (33%) were suffering from colon cancer followed by pancreatic cancer (19%). The remaining patients were suffering from sigmoid, bile duct, gastric, esophagus, breast and cervical cancer. Most patients

TABLE 1 Patient characteristics

	Patient group (n = 27)	Men (n = 16)	Woman (n = 11)	Chemotherapeutic drugs	Number of patients
Age (yr)					
Mean ± SD	61 ± 10	63 ± 9	58 ± 11		
Range	40–78	51–78	40–72		
Cancer localization					
Colon	9	5	4	Capecitabine	7
				5FU/LV	1
				5FU, Oxaliplatin	1
Pancreatic	5	2	3	5FU/LV, Oxaliplatin, Lapatinib	5
Sigmoid	3	3	0	Capecitabine	1
				5FU/LV, Oxaliplatin, Lapatinib	1
				5FU/LV, Irinotecan	1
Bile duct	3	3	0	5FU/LV, Oxaliplatin, Lapatinib	3
Gastric	2	2	0	Capecitabine	1
				5FU/LV, Oxaliplatin, Lapatinib	1
Esophagus	2	1	1	Capecitabine	1
				5FU/LV, Oxaliplatin, Lapatinib	1
Breast	2	0	2	Capecitabine	2
Cervical	1	0	1	5FU/LV, Oxaliplatin, Lapatinib	1
DPD activity (nmol/mg/h)					
PBM cells	8.8 ± 2.6	8.5 ± 2.2	9.1 ± 3.2		
Mean ± SD Range	5.1–15.1	5.9–12.7	5.1–15.1		

LV, leucovorin

were treated with capecitabine (44%) or 5FU/LV combined with oxaliplatin and lapatinib (44%).

With respect to the toxicities encountered in the patients upon treatment with 5FU-based chemotherapy, 4 patients suffered from grade I/II hematological toxicity and 4 patients suffered from grade III/IV hematological toxicity. Grade I/II and grade III/IV gastrointestinal toxicity was observed in 19 patients and 4 patients, respectively. Grade I/II and grade III/IV flu-like symptoms were observed in 10 and 3 patients, respectively. With respect to dermatological toxicity, grade I/II and grade III/IV toxicity was observed in 16 and 1 patient, respectively.

In the group of patients treated with capecitabine or 5FU combined with other chemotherapeutic drugs, no significant correlations were observed in DPD activity and the severity of gastrointestinal toxicity or flu-like symptoms. However, the DPD activity was higher in patients experiencing grade I/II hematological toxicity (11.2 ± 1.7 nmol/mg/h) compared to those without hematological toxicity (8.4 ± 2.3 nmol/mg/h) (Figure 1A). In addition, the DPD activity in patients with grade I-II dermatological toxicity including the hand-foot syndrome (7.9 ± 1.9 nmol/mg/h) was significantly lower when compared to Grade 0 (10.4 ± 3.0 nmol/mg/h; $P = 0.016$) (Figure 1B). Furthermore, in this group of patients, the DPD activity in patients with grade I/II hand-foot syndrome (7.8 ± 1.7 nmol/mg/h, $n = 9$) was lower when compared to those without the hand-foot syndrome (9.4 ± 2.9 nmol/mg/h, $n = 17$) although not yet significant ($P = 0.1$).

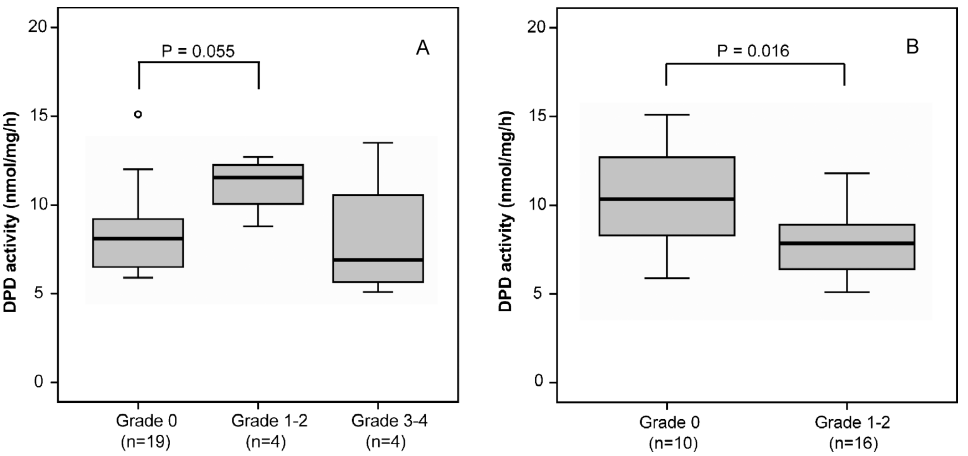

FIGURE 1 Box plots of the DPD activity in patients treated with capecitabine or 5FU combined with other chemotherapeutic drugs. The top, bottom and line through the middle of a box correspond to the 75th, 25th, and 50th percentile, respectively. The whiskers on the bottom extend from the 5th and top 95th percentile. The open circle represent an outlier. The distribution of the DPD activity is indicated for patients with hematological toxicity (panel A) and dermatological toxicity (panel B).

DISCUSSION

In this study, we have investigated the relationship between the DPD activity in PBM cells and the degree of toxicity experienced by cancer patients treated with 5FU-containing chemotherapy. Patients with a partial DPD deficiency have an increased risk of developing grade IV neutropenia which might be caused by increased levels of fluoropyrimidine nucleotides, the anabolic products of 5FU.[14] In our patient group, only one patient suffered from grade IV neutropenia and the DPD activity in PBM cells (5.1 nmol/mg/h) of this patient was comparable to that observed for patients with a partial DPD deficiency.[14] Previously, we showed that in patients treated with only 5FU/LV, the DPD activity in PBM cells of patients experiencing mild grade I/II neutropenia was increased when compared to the DPD activity in PBM cells of patients without neutropenia and those suffering from grade III/IV neutropenia.[15] In this study, we showed that a similar phenomenon was observed in patients treated with capecitabine or 5FU combined with other chemotherapeutic drugs. In this respect, it is worthwhile to note that the downstream catabolites of 5FU have been associated with toxicity. A patient with a partial dihydropyrimidinase deficiency and thus, a decreased capacity to degrade fluoro-5,6-dihydrouracil (FUH_2) suffered from severe toxicity including leucopenic fever.[16] In rats, FUH_2 and fluoro-ß-alanine (FBAL) attenuated the antitumor activity and increased the toxicity of 5FU.[17,18] Furthermore, a pharmacokinetic analysis showed a positive correlation between the AUC of FBAL and grade 3-4 diarrhea.[19] Thus, mild neutropenia (grade I/II) might be associated with increased concentrations of the catabolic products of 5FU and therefore, an increased activity of DPD.

The mean DPD activity in PBM cells was significantly lower in patients suffering from dermatological toxicity, including the hand-foot syndrome, when compared to patients without such toxicities. Rash is a common lapatinib-related adverse event whereas the hand-foot syndrome is the most frequently encountered adverse event observed after treatment of patients with capecitabine or 5FU administered via continuous infusion,[2,20] Up to 60% of all patients treated with capecitabine suffered from some form of hand-foot syndrome, with 17% of the patients suffering from severe grade III/IV toxicity.[2] The observation that the hand-foot syndrome was hardly encountered in patients treated with oral fluoropyrimidines combined with an inhibitor of DPD, might suggest that the catabolic products of 5FU are responsible for the onset of the hand-foot syndrome.[21] However, pharmacokinetic analysis of some metabolites of capecitabine, such as 5FU and FBAL, failed to demonstrate a correlation between the plasma concentrations and the occurrence or severity of the hand-foot syndrome.[19,22] Nevertheless, our study indicates that patients with a low-normal DPD activity are prone to develop dermatological toxicity and the hand-foot syndrome

suggesting that the increased availability of 5FU for the synthesis of fluoropyrimidine nucleotides can give rise to the onset of the hand-foot syndrome. Thus, patients with a low-normal or high-normal DPD activity proved to be at risk of developing mild toxicity upon treatment with 5FU-based chemotherapy, demonstrating the important role of DPD in the etiology of toxicity associated with 5FU and the catabolites of 5FU.

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