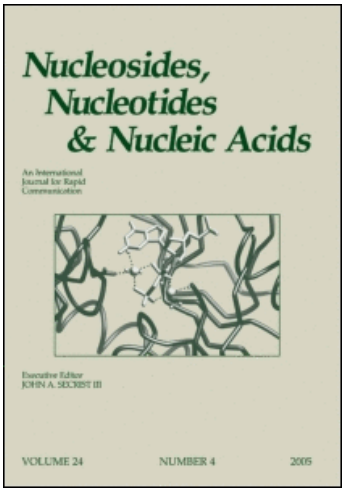

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Pyrimidine Degradation Defects and Severe 5-Fluorouracil Toxicity

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ABSTRACT

5-Fluorouracil (5FU) remains one of the most frequently prescribed chemotherapeutic drugs for the treatment of cancer. Recently, the pivotal role of the catabolic pathway of 5FU in the determination of toxicity towards 5FU has been highlighted. Patients with a (partial) dihydropyrimidine dehydrogenase deficiency proved to be at risk of developing severe toxicity after the administration of 5FU. A partial dihydropyrimidinase deficiency proved to be a novel pharmacogenetic disorder associated with severe 5FU toxicity.

Key Words: Dihydropyrimidine dehydrogenase; 5-Fluorouracil; Dihydropyrimidinase; DPYD; DPYS; Toxicity.

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INTRODUCTION

5-Fluorouracil (5FU) is one of the most commonly used chemotherapeutic agents for the systemic treatment of cancers arising from the gastrointestinal tract, breast and head and neck. A challenging field is the identification of patients with an increased risk of development of severe 5FU-associated toxicity. It would allow either dose-adaptation or the application of new non-fluoropyrimidine based chemotherapeutic drugs. A meta-analysis involving 1,219 patients with colorectal cancer showed that grade 3 to 4 toxicity was encountered in 31–34% of the patients receiving 5FU, with 0.5% of the patients experiencing lethal toxicity.[1] It is likely that a significant proportion of these adverse drug reactions are due to genetically based differences, between individuals, in the response to 5FU. Recent advances in our understanding of the metabolism of 5FU and the key-enzymes involved in the activation and degradation of 5FU has led to an increased awareness that the catabolic route of 5FU plays an important role in the determination of toxicity towards 5FU.[2,3]

CATABOLISM OF 5FU

Dihydropyrimidine dehydrogenase (DPD) is the initial and rate-limiting enzyme in the catabolism of 5FU ans it catalyses the conversion of 5FU to fluoro-5,6-dihydrouracil (FUH_2) (Fig. 1). FUH_2 can be further degraded to fluoro-β-ureidopropionate (FUPA) and subsequently to fluoro-β-alanine (FBAL) by dihydropyrimidinase and β-ureidopropionase, respectively.

DPD DEFICIENCY

The activity of DPD is a critical factor with respect to the pharmacokinetics of 5FU and clinical toxicity. 5FU has a relatively narrow therapeutic index and a strong correlation has been described between exposure to 5FU and both haematological and gastrointestinal toxicity. Patients with a partial or complete DPD deficiency have a decreased capacity to degrade 5FU and are at risk of developing severe 5FU-associated toxicity.[4,5] The importance of a DPD deficiency in the etiology of unexpected severe 5FU toxicity has been demonstrated by the fact that in 39–61% of the cases, a decreased DPD activity could be detected in Peripheral Blood Mononuclear cells.[3,6,7] Patients with a partial DPD deficiency proved to have a 3.4 fold higher risk of developing grade IV neutropenia than patients with a normal DPD activity.[6,8] Furthermore, in patients with a low DPD activity, the onset of toxicity occurred, on average, twice as fast compared to patients with a normal DPD activity.[3]

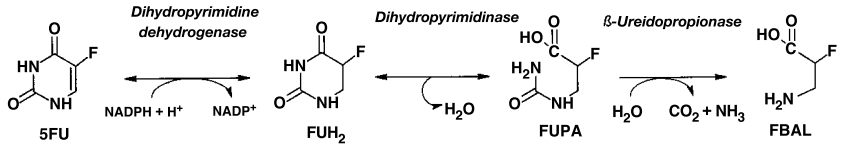

Figure 1. The catabolic route of 5FU.

DPD GENE

The human DPD gene (*DPYD*) is present as a single copy gene on chromosome 1p22 and consists of 23 exons.[9] A physical map indicates that *DPYD* is at least 950 kb in length with 3 kb of coding sequence and an average intron size of about 43 kb.[9] To date, 32 different mutations and polymorphisms have been identified in *DPYD* (Fig. 2). In patients suffering from severe toxicity after the administration of 5FU, the presence of mutant *DPYD* alleles have been demonstrated.[2–4,6,8,10] In this group of patients, 12 mutations have been identified including 1) one splice site mutation (IVS14 + 1G > A); 2) two nonsense mutation (R21X, E386X); 3) 4 missense mutations (M166V, V335L, I560S and D949V) and 5 polymorphisms (C29R, R21Q, S534N, I543V, V732I).[2] Analysis of the prevalence of the various mutations among cancer patients suffering from severe 5FU-associated toxicity showed that the IVS14 + 1G > A mutation is the most common one and could be detected in 28% of all patients suffering from grade 3–4 5FU-associated toxicity.[8,11]

DIHYDROPYRIMIDINASE DEFICIENCY AND 5FU TOXICITY

In addition to DPD, it has also been suggested that patients with a deficiency of dihydropyrimidinase (DHP) are at risk of developing severe 5FU-associated toxicity.[12–14] DHP deficiency is an autosomal recessive disease characterised by dihydropyrimidinuria and has been associated with a variable clinical phenotype.[15]

Recently, we demonstrated for the first time that in one patient the severe toxicity, after a treatment with 5FU, was attributable to a partial deficiency of DHP.[16] Analysis

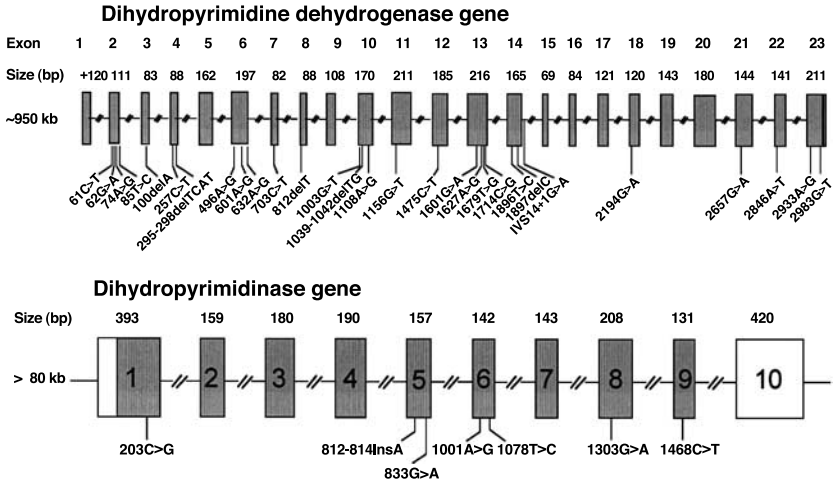

Figure 2. Genomic organisation of the DPD and DHP gene. The DPD gene (*DPYD)* consists of 23 exons with an open reading frame of 3075 bp (upper panel). The DHP gene (*DPYS*) consists of 10 exons encoding an open reading frame of 1560 bp (lower panel). The different mutations identified in patients with a deficiency of DPD or DHP are indicated, numbers correspond to the cDNA position.

of the DHP gene showed that the patient was heterozygous for the missense mutation 833G > A (G278D) in exon 5 (Fig. 2). Heterologous expression of the mutant enzyme in *Escherichia coli* showed that the G278D mutation leads to a mutant DHP enzyme without residual activity. An analysis for the presence of this mutation in 96 unrelated Dutch Caucasians indicates that the allele frequency in the normal population is less then 0.5%.[16]

Under normal conditions, a low DHP activity is probably sufficient to maintain dihydrouracil and dihydrothymine homeostasis as heterozygotes do not excrete elevated levels of dihydropyrimidines. After the loading of such patients with uracil, the accumulation of dihydrouracil in urine increased several fold compared with normal individuals, indicating a decreased capacity of heterozygotes to degrade dihydropyrimidines.[14] In this respect, it is worthwhile to note that the co-administration of FUH_2 with 5FU attenuated the antitumor activity and increased the toxicity of 5FU.[17] Our results showed that a partial DHP deficiency is a novel pharmacogenetic disorder associated with severe 5FU toxicity.

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