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Correlation between dihydropyrimidine dehydrogenase and efficacy and toxicity of fluoropyrimidine drugs

X.-Q. LIU[1,2], M. ZHUANG[1], Z. WANG[2], R.M. HUBER[2]

[1]Department of Medical Oncology, No. 1 Renmin Hospital of Lianyungang, Lianyungang, P.R. China
[2]Division of Respiratory Medicine and Thoracic Oncology, Department of Internal Medicine V, Thoracic Oncology Centre Munich, Ludwig-Maximilians Universität München, Germany

Abstract. **At present, fluoropyrimidine, based on 5-fluorouracil (5-FU), remains one of the most frequently prescribed chemotherapeutics drugs for the treatment of cancer. Dihydropyrimidine dehydrogenase (DPD) is the rate-limiting enzyme in the catabolism of 5-FU, and DPD enzymatic activities are usually varied dramatically from individual to individual, including both the intrapatient differences and the interpatient variability. There is a certain correlation between the DPD activity and efficacy and toxicity following the administration of fluoropyrimidine drugs. Partial or complete loss of DPD activity can lead to serious or even lethal toxicity. In this article, we review the relationship between DPD activity and efficacy and toxicity following the administration of fluoropyrimidine drugs, and also the structure, function, and characteristics of DPD.**

We report here that measurement of DPD activity may become a strategy and be paid much attention to predict the efficacy and toxicity prior to starting a fluoropyrimidine-based therapy.

Key Words:
Dihydropyrimidine dehydrogenase, Fluoropyrimidine drugs, Efficacy, Toxicity.

Introduction

The fluoropyrimidine drugs have been widely applied to treatment of antitumor including 5-fluorouracil (5-FU), tegafur, compound tegafur Tegafur-Uracil, Carmofur, floxuridine, deoxy-fluorouridine, chemotherapy-targeted capecitabine, S-1 (firstly appearing on the market in Japan), and Tiji'ao capsule (made in China), among of which 5-FU has been firstly conducted in clinical trial and the other drugs are derivatives from 5-FU. These drugs *in vivo* need to be converted into 5-FU and play a role in the treatment of cancer through the natural hydrolysis and metabolism by the P450 enzymes or by a specific enzyme system in tumor tissue. 5-FU *in vivo* is converted into 5-fluoro-2-deoxyuridine nucleotides, which inhibit the thymine nucleotide synthase and prevent transformation of the deoxyuridine nucleotide into the deoxy-thymidine nucleotides, accordingly suppressing DNA synthesis. In addition, inhibiting RNA synthesis can be achieved by preventing uracil and orotic acid incorporated into RNA. These kinds of drugs have been widely used in chemotherapy of a variety of malignant tumors such as gastrointestinal tumors, head and neck tumors, and breast tumors, in which toxicity is mainly displayed as gastrointestinal reaction, oral mucositis, bone marrow suppression, nervous system toxicity, and so on. Clinical trials, thus, far demonstrate that under the same treatment condition there is a great variability for different patients related to efficacy and toxicity of anticancer drugs. In recent years, scientists have reported that the DPD enzymes play an important role in determination of the efficacy and the toxicity of 5-FU drugs. Individual differences of DPD enzyme activity is one of the most important reasons resulting in individual variability of efficacy and toxicity treated by 5-FU drugs.

Structure, Function and Mechanism of DPD

Structure of DPD

DPD is an enzyme encoded by the dihydropyrimidine dehydrogenase gene (*DPYD*), which is located on the short (p) arm of chromosome 1 at position 22 (1p22) containing 23 exons (about 950 kb)[1,2]. The wild-type *DPYD* gene encodes a protein composed of 1025 amino acid with two identical subunits and a molecule (M_w = 150 kD)[3] containing binding sites with nicotinamide adenine dinucleotide phosphate, flavin adenine dinucleotide, and pyrimidine alkali.

Corresponding Author: Rudolf M. Huber, MD; e-mail: Huber@med.uni-muenchen.de

Acting Mechanism of DPD

The enzyme kinetic studies have shown that DPD takes effect by nonclassical two sites ping-pong kinetic mechanism: combination of two separate sites with NADPH/$NADP^+$ and pyrimidine alkali or 5-FU, respectively. After reduction of FAD using NADPH as hydrogen donor, electrons are most likely transferred to FMN via [4Fe-4s] cluster, resulting in reduction of pyrimidine alkali[4]. 5-FU is reduced to the dihydrofluorouracil (FUH_2). After ring cleavage through dihydropyrimidinase, 5-fluoro-β-ureide propionic acid (FUPA) is produced, which is finally synthesized into 5-fluoro-β-alanine (FBAL) under catalysis of β-alanine synthase and excreted by the kidney.

Clinical Manifestations and Distribution Feature of DPD Deficiency

Clinical DPD Deficiency

Clinical DPD deficiency is observed with both the phenotypes and genotypes: (1) phenotype is the apparent abnormal birth after onset, manifested as spasms, bradykinesia, and mental retardation; (2) genotype is the usually asymptomatic, only performing severe toxicity during chemotherapy treated by fluorinated pyrimidine drugs[5]. These two kinds of manifestations are based on different molecular genetics. In 1985, Tuchman et al[6] first reported that a breast cancer patient, having induced by severe diarrhea, myelosuppression, neurotoxicity, and disturbance of consciousness, was treated by regular 5-FU chemotherapy. Detectable pyrimidine concentrations in the blood and urine samples of patient increased abnormally, strongly suggesting the DPD activity deficiency. Thereafter, there have been reports on toxicity due to partial or complete lacking of DPD activity during chemotherapy treated by 5-FU. Its toxicity is mainly manifested as the gastrointestinal tract and bone marrow suppression, but severer (mostly grade IV than patients with normal enzyme activity, even it is hard to avoid toxicity by dose reduction. In addition, neurotoxicity in patients with DPD activity deficiency is relatively common. In 1999, Milano et al[7] reported that, among the whole group of 11 patients with DPD activity deficiency, seven had significant neurotoxicity, and leading to patient death in two cases, moreover pointing out that women are particularly prone to DPD enzyme deficiency. Typical neurotoxic patients, having some neurological symptoms of slow onset or rapid emergence of epilepsy and disturbance of consciousness, are performed with the cerebellar ataxia and encephalopathy.

Distribution Feature of DPD

DPD is widely distributed in most tissue of people, especially exhibiting the highest activity in the liver tissue and peripheral blood mononuclear cells (PBMC). In addition, it can also be found in the tumor and inflammatory tissue. The DPD activity distribution in PBMCs shows a Gaussian distribution, but a large degree of interindividual variation is observed, even up to 5-26 times. A meta-analysis of over 1200 cases of patients showed that more than 30% of patients suffered severe drug-related toxicity after chemotherapy using 5-FU[8]. It was estimated that the incidence of low DPD activity among the total population was about 3-5%[9,10]. It was also reported that differences of DPD activity among different ethnic groups have been noted. Phenotypic and genotypic analysis showed that, among East Asia (Japanese, Taiwanese, South Korean), South Asia (Indian, Pakistani, Sri Lankan), Africa (Egyptian, Kenyan, Ghanaian), Europe (whites), and the United States (whites and African-American), the incidence of low DPD activity remains variable, for example, the South Koreans have a higher level of DPD enzyme activity while a higher incidence rate of low DPD activity in African-American women can be observed[2,11-16]. At present, the definition of low DPD activity threshold value has not yet reached a consensus. Prior to using the fluoropyrimidine drugs, as a treatment in cancer, more attention should be paid on how much of lowest DPD activity it can reach, which is not known and needs to be further studied.

Methodology of DPD Assay

There are several techniques for detecting the DPD in blood and tissue at a gene or protein level. The most common approach is to measure both the dihydrouracil (UH_2) and the uracil (U) concentrations in plasma by a high-performance liquid chromatography (HPLC) and to evaluate the DPD activity *in vivo* by UH_2/U ratio. This method is simpler and accurate for DPD activity assay comparing to the radio-enzymatic analysis requiring large volume of radio substrates and time. Although the highest DPD enzyme activity is known in the liver, it is difficult to obtain liver sample from patient and is seldom used. A new technique was reported to speculate the DPD en-

zyme activity by quantifying peripheral DPYD mRNA according to a real-time quantitative RT-PCR Reverse transcription polymerase chain reaction. However, there is a conflict among the recent several studies and conclusions[17-21], and whether replacing DPD enzyme activity assay by quantification of peripheral DPYD mRNA still needs to be further explored. It was also reported[22] that the final metabolite of 5-FU, FBAL, may be used to predict the DPD activity *in vivo*, showing that low FBAL level indicates the low DPD activity. In 2004, Mattison et al[23] reported a novel, developed, noninvasive, testing method. After oral administration of 2-^{13}C-uracil, $^{13}CO_2$ and $^{12}CO_2$ level in exhaled breath were detected by using IR spectroscopy, and it was concluded that decreasing $^{13}CO_2$ in exhaled breath was associated with partial or complete deficiency of DPD activity.

Relationship Between DPD Gene Polymorphism and its Decreased Activity

DPD is encoded by the DPYD, in which nucleotide mutation may cause changes of DPD structure and activity. To date, genetic polymorphism of DPD encoded by DPYD has been gradually reported. More than 40 mutation sites have been identified and occurrence frequency in some sites has been studied too. The splice-site mutation IVS14+1G > A was firstly found in DPYD gene sequence variant, which is the result of GT to AT alternation in the nucleotide at the exon 14 acceptor splice site leading to lacking of 165 bp segment in exon 14 and skipping of exon 14 immediately up-stream of the mutated splice donor site. As a result, the DPD activity is decreased markedly. Kuilenburg et al[5] investigated that among the mutations responsible for DPD activity decreasing and deficiency, the IVS14+1G > A mutation was mostly occurred showing a prevalence in Nordic populations. Jia et al[24] have investigated the polymorphism of three mutation sites on *DPYD* gene (IVS14+1G → A, Exon13 A1627G, Exon11 G1156T) in Chinese Han ethinicity groups. The results showed that the polymorphism of Exon13 A1627G mutation site is present in Chinese Han ethinicity groups, while there is no polymorphism for the IVS14+1G → A and Exon11 G1156T mutation sites. Some of the mutations, such as A1627G and G2194A sites, do not cause a decreased DPD activity. Analysis on polymorphism of these mutation sites and relationship between DPD activity and mutation of these sites needs to be explored in depth.

Correlation Between DPD and Efficacy and Toxicity of Fluoropyrimidine Drugs

Antitumor Mechanism of Fluoropyrimidine Drugs

Currently, in addition to the traditional 5-FU, capecitabine and S-1 (also called Tiji'ao capsule, made in China) have been widely used in the treatment of cancer. Capecitabine (namely, *n*-4-pentyloxycarbonyl-5′-deoxy-5-fluorocytidine, CAP), belonging to a thymidylate synthase (TS) inhibitor group, is a prodrug chemically synthesized by FU. It can be absorbed rapidly through the intestinal mucosa as an intact molecule due to containing urethane structure. CAP is a type of oral-administrated fluoropyrimidine nucleoside drugs with targeted effect. It can be selectively activated in tumor tissue and produce high concentrations of the active cell toxic substances, thereby improving the tolerance of cancer patients and maximizing the anticancer activity. S-1 firstly appeared on the market in Japan and applied in clinical trials in 1994. It has now been approved for the treatment of advanced gastric cancer, head and neck cancer, colorectal cancer, and metastatic breast cancer. S-1 is an oral anticancer drug with compound ingredients, composed of tegafur, gimestat, and otastat potassium in a molar ratio of 1:0.4:1, of which gimestat can selectively inhibit the hepatic DPD activity and prevent the catabolism of 5-FU *in vivo*, maintaining 5-FU at a higher concentration in plasma and tumor tissue and prolonging the half-life of 5-FU, while otastat potassium is also an inhibitor that is intended to mitigate the 5-FU-related gastrointestinal toxicity by preventing the phosphorylation of 5-FU. In recent years, there has been reported to cause severe toxicity during chemotherapy treated by the S-1, because the gimestat inhibits DPD activity, thereby resulting in severe toxicity for patients with DPD deficiency.

Relationship Between DPD and Efficacy of Fluoropyrimidine Drugs

In 1999, Ishikawa et al[25] and Kirihara et al[26] reported that, in tumor cells *in vitro*, gene expression and activity of DPD were related to the therapy sensitivity of 5-FU. High DPD mRNA expression and activity level may give rise to resistance to 5-FU drugs, otherwise leading to severe toxicity. Dong et al[27] have studied the relationship between DPD level and serum concentration of 5-FU, efficacy and toxicity in colorectal can-

cer patients. DPD level and serum concentration of 5-FU significantly vary among the different colorectal cancer patients. DPD level is negatively correlated with serum 5-FU concentration and toxicity, while serum concentration of 5-FU is positively related to adverse response and treatment efficacy, which correspond well to most conclusions reported previously. Horiguchi et al[28] investigated DPD expression and prognosis in a total of 191 patients with invasive breast cancer treated by 5-FU, showing that patients with DPD expression-positive tumors had a significantly poorer prognosis in disease-free and overall survival compared to those with DPD-negative tumors ($p < 0.05$). But, the efficacy of 5-FU was correlated with the other enzyme (such as TS) expression involved in metabolism. In addition, DPD activity may be regulated by post-transcriptional level, which needs to be further studied.

Relationship Between DPD and Toxicity of Fluoropyrimidine Drugs

A reduced DPD activity will result in increasing serum concentrations of 5-FU drugs, thus, increasing the risk of patients suffering from severe toxicity. Van Kuilenburg et al[29] demonstrated that in 59% of the cases (22 of 37 patients suffering from severe toxicity after administration of 5-FU drugs), DPD activity deficiency can be detected in PBMCs. Comparing the two groups of patients with a low DPD activity and a normal DPD activity, no differences in hematological, gastrointestinal, or flu-like symptoms were observed between both groups, with the exception of grade IV neutropenia, indicating that reducing DPD activity will significantly suppress bone marrow. Dong et al[27] have studied serum levels of DPD distributed in 72 patients, showing that UH_2/U ratio is negatively correlated with serum concentration of 5-FU. UH_2/U ratio in patients suffered from grades II-IV oral mucositis and diarrhea is lower than that in patients with grades 0-I.

Conclusions

DPD plays an important role during chemotherapy treated by 5-FU drugs. Detecting and assessing DPD activity may become a strategy to improve the efficacy and avoid the severe toxicity prior to starting a fluoropyrimidine-based therapy. At present, it is imperative for comprehensive understanding the variability of individual tolerance to fluoropyrimidine drugs, so the potential patients with severe toxicity can be treated with lower initial doses of chemotherapy drugs to avoid the occurrence of severe toxicity. Along with the emergence of personalized chemotherapy era, it is believed that the patients can be treated according to different tumor identity card, which is now within our grasp.

Conflict of interest

The Authors declare that they have no conflict of interests.

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