Clinical Implications of Dihydropyrimidine Dehydrogenase on 5-FU Pharmacology

By Robert B. Diasio, MD [2]

Dihydropyrimidine dehydrogenase (DPD) is the initial rate-limiting enzyme in the catabolism of 5-fluorouracil (5-FU), accounting for catabolism of over 85% of an administered dose of 5-FU. DPD plays an important role in

Introduction

Dihydropyrimidine dehydrogenase (also known as DPD, dihydouracil dehydrogenase, dihydrothymine dehydrogenase, uracil reductase, EC 1.3.1.2) is the initial rate-limiting enzymatic step in the catabolism of not only the naturally occurring pyrimidines, uracil and thymine, but also the widely used antimetabolite cancer chemotherapy drug, 5-fluorouracil (5-FU).[1,2] As shown in Figure 1, DPD occupies an important position in the overall metabolism of 5-FU, converting over 85% of a standard dose of administered 5-FU to dihydrofluorouracil (5-FUH₂), an inactive metabolite, in an enzymatic step that is essentially irreversible.[3]

It is true that anabolism is clearly critical for 5-FU cytotoxic action through conversion of 5-FU to the "active" nucleotides 5-fluoroxyouridine monophosphate (FdUMP), 5-fluorouridine triphosphate (FUTP), and 5-fluoroxyouridine triphosphate (FdUTP). These important active metabolites are, in turn, responsible for inhibition of cell replication through inhibition of thymidylate synthase, or through incorporation into RNA or DNA, respectively. Nevertheless, it is pyrimidine catabolism through the rate-limiting step, DPD, that controls the availability of 5-FU for anabolism and thus occupies a critical position in the overall metabolism of 5-FU.

Evidence of Importance of DPD Activity to 5-FU Pharmacology

DPD as the rate-limiting step in pyrimidine catabolism has recently been shown to play a critical role in determining the clinical pharmacology of 5-FU (Table 1). In particular, it has been demonstrated that DPD accounts for much of the variability that has been noted in clinical studies with 5-FU. This includes both intrapatient variability, as well as interpatient variability.

DPD activity has been observed to follow a circadian pattern in both animals and humans.[4-6] Studies in rats on a 12-hour light/12-hour dark schedule have demonstrated that hepatic levels of DPD follow a pattern that can be plotted on a cosine wave.[4] This pattern was completely reversed in another group of rats on an inverted 12-hour light/12-hour dark schedule. In patients receiving continuous-infusion 5-FU by automated pumps, sampling DPD activity in peripheral blood mononuclear cells over a 24-hour period has also been shown to exhibit circadian patterns when plotted on a cosine wave.[6] Serum samples obtained at the same time from the same patients have been shown to have serum 5-FU concentrations that were also characterized by a circadian pattern, which was essentially inverse to the DPD circadian pattern (Figure 2).

Data from this study suggested that perhaps DPD was responsible for the circadian variation in 5-FU, leading some chemotherapists to propose time-modified 5-FU infusions to optimize drug delivery during a 24-hour period. Such regimens have been suggested by some oncologists, particularly those in Western Europe, to have a potential benefit in the treatment of certain human cancers.[7]

What Causes the Variation?

For the past 4 decades, it has been unclear as to why the pharmacokinetics of 5-FU have been so variable, with half lives (t₁/₂) ranging from around 4 minutes to 25 minutes after an intravenous bolus...
Because of the critical position of DPD in the metabolic pathway, it was hypothesized that the variation in pharmacokinetic characteristics might be secondary to variability of DPD enzyme activity among different individuals. Population studies were undertaken assessing DPD enzyme activity initially in tissues (peripheral blood mononuclear cells and liver) from healthy individuals. DPD was shown to vary from individual to individual, with a normal distribution pattern (bell-shaped curve) showing a six-fold variation from the lowest to the highest values (Figure 3).[9,10]

Essentially the same pattern of DPD activity has also been observed in the peripheral blood mononuclear cells of both breast and colorectal cancer patients, although, interestingly, the normal distribution is shifted to the left, with lower median DPD activity in the breast cancer patient population.[11] The mechanism for this latter observation is unknown. The wide variation in DPD activity observed in the populations described above is thought to account for the wide variation in the half-life (and pharmacokinetics) observed in patients treated with 5-FU.[8]

### Variation in DPD Activity

While most patients tolerate 5-FU reasonably well, over the past 4 decades, a number of patients have developed severe, at times life-threatening, toxicity after standard doses of 5-FU.[12-15] Because these patients demonstrated exaggerated normal toxic side effects (as if they had received an overdose of drug), it was hypothesized that these patients were deficient in a catabolic enzyme that resulted in more 5-FU being present over time.

Initial studies in these affected patients demonstrated elevated uracil and thymine levels, suggesting a deficiency in the first step in pyrimidine catabolism, DPD.[12-14] Subsequent studies demonstrated that many of these patients were indeed DPD deficient. It is now clear that an additional small percentage (< 3%) of such patients have DPD activity that is significantly below the normal distribution seen in the average person.[15] These individuals are at significant risk if they develop cancer and are given 5-FU. This is a true pharmacogenetic syndrome, with symptoms being unrecognizable until exposure to the drug.[15]

Variation in DPD activity has also recently been shown to be responsible for the apparent variable bioavailability of 5-FU, which over the years has led to the recommendation that 5-FU not be administered by the oral route. An explanation for the erratic bioavailability of 5-FU has previously been unclear, particularly since 5-FU is a small molecule with a $pK_a$ that should favor excellent absorption and bioavailability. Recent studies using DPD inhibitors in animals have demonstrated that, following inhibition of DPD, the pharmacokinetic pattern resulting from oral administration of 5-FU is essentially the same as that produced by intravenous administration, resulting in essentially 100% bioavailability.[16]

While it has long been well known that variability in the activity of pyrimidine anabolic enzymes is important in determining the antitumor effectiveness of 5-FU, much less attention has focused on the variability in activity of the pyrimidine catabolic enzymes. Tumors that are resistant to 5-FU have been shown to express increased levels of DPD activity.[17] With the development of the quantitative polymerase chain reaction (PCR) to measure DPD mRNA, increased expression of DPD mRNA has been demonstrated in tumors of patients who were resistant to 5-FU.[18]

### Developing a Pharmacologic Strategy Based on DPD

The variability in DPD levels in both normal and tumor tissues provides a basis for the chemotherapist to consider either altering the dose of the fluoropyrimidine drug or inhibiting DPD in order to eradicate the variability in 5-FU pharmacology. The presence of increased tumor DPD could lead to a decision to increase the dose of 5-FU (or a 5-FU prodrug) to overcome the increased catabolism of 5-FU within the tumor, thus increasing the amount of 5-FU that could be converted to active anabolites (Figure 1).

An alternative approach is to use known inhibitors of DPD, usually with a lower dose of 5-FU (or the use of a 5-FU prodrug; eg, capecitabine [Xeloda]), to directly inhibit 5-FU degradation within the
tumor, thereby permitting the existing 5-FU (even if present in low concentrations) to be converted to active anabolites. Inhibiting DPD in 5-FU-susceptible host tissue, such as gastrointestinal mucosa and bone marrow, should also make dosing from patient to patient less variable, the latter being accomplished through lessening the variability in pharmacokinetics, bioavailability, and the resultant host toxicity.

**Dihydropyrimidine Dehydrogenase Inhibitory Fluoropyrimidines**

Over the years, there have been many attempts to synthesize effective inhibitors of DPD.[19] Unfortunately, many of these compounds have proven to be very toxic. In the past several years, several fluoropyrimidine drugs using DPD inhibition have been introduced into the clinic. These drugs, referred to as dihydropyrimidine dehydrogenase inhibitory fluoropyrimidines (DIF), include uracil and tegafur (UFT), eniluracil (GW-776C85; 5-ethynyluracil), S-1, and emitefur (BOF-A2), all of which have recently been in clinical studies.[20] These drugs differ both in type of DPD "inhibition," as well as degree of inhibition produced.

The rationale for using DIF drugs is that they are a source of 5-FU, either from 5-FU itself or from a "prodrug" that is converted to 5-FU, combined with another agent that interferes with (or inhibits) the otherwise rapid catabolism of 5-FU. This permits oral delivery of 5-FU (bioavailability > 70%) and results in less variability in the pharmacokinetics of the fluoropyrimidines. In addition, by inhibiting the catabolic pathway, more 5-FU can enter the anabolic pathway, thereby potentially increasing the antitumor effect. This is theoretically important for tumors that are resistant secondary to an increase of intratumoral DPD. Lastly, while not completely understood, it is thought that some 5-FU toxicities (hand-foot syndrome; some forms of neurotoxicity; and possibly, cardiotoxicity) may be secondary to the catabolic pathway. Inhibiting the catabolic pathway might decrease the incidence of these toxicities.

**UFT: The Most Studied Dehydrogenase Inhibitory Fluoropyrimidine**

UFT was the first DIF drug to be synthesized; it is also the most studied DIF drug.[21,22] This "new" fluoropyrimidine is a combination of the naturally occurring pyrimidine, uracil, with the fluoropyrimidine, tegafur (ftorafur), in a 4:1 molar ratio. The presence of uracil in excess of 5-FU results in "competition" at the level of DPD, such that the 5-FU, which is formed from tegafur, will not be rapidly degraded and will remain present for a prolonged period. While this is not a true "inhibition" of DPD, the competition between 5-FU and uracil for DPD produces an effect similar to that achieved with a true DPD inhibitor.

In contrast to the true DPD inhibitors and inactivators, the effect on DPD is more rapidly reversible. This rapidly reversible inhibition may avoid some of the problems seen with the earlier DPD inhibitors, and may also account for a more favorable toxicity profile compared to some of the earlier DPD inhibitors,[19] as well as some of the newer DIF drugs.

Extensive data from Japan, as well as Europe, South America, and the US, now demonstrate that orally administered UFT has antitumor activity in several tumor types (particularly breast and colon cancer), either as a single agent or combined with leucovorin.[23] Studies thus far have shown that it is at least as effective as intravenously infused 5-FU. Furthermore, its toxicity profile has proven quite tolerable, with the typical fluoropyrimidine toxicities (eg, diarrhea and nausea) seen at the maximum-tolerated dose. Of note is the paucity of other toxicities, in particular hand-foot syndrome, neurologic, and cardiotoxicity.[24] Although not well understood, these toxicities may be secondary to 5-FU catabolites. Such catabolites are less likely to form from UFT; therefore, these toxicities are not typically observed.

**Conclusion**

While DPD is clearly an important factor,[25] a more rational approach would probably be to monitor multiple factors that are known to predict 5-FU effectiveness. These might include not only DPD, but also the target thymidylate synthase, and possibly the enzymatic steps leading to anabolism of 5-FU to active nucleotide anabolites. One could then base the modulation of 5-FU on the total picture. In
particular, immunohistochemical evaluation or quantitative PCR of multiple targets may provide a valuable approach to such assessment.

Recently, patients in a clinical trial for advanced colorectal cancer were assessed for response to 5-FU plus leucovorin (Mayo Clinic Regimen) and independently assessed for levels of DPD, thymidylate synthase, and thymidine phosphorylase. Such assessment proved quite accurate in predicting response to therapy. Patients with relatively "low" expression of all of these markers responded to the regimen, while those patients who showed elevation of even one marker were unresponsive (Figure 4). It is likely that this type of approach, using assessment of multiple markers, will become the standard of care in the future.

Questions and Answers

Daniel Haller, MD: This questions concerns dihydropyrimidine dehydrogenase (DPD) activity and Dr. Diasio’s statement that the normal distribution is shifted to the left, with lower median DPD activity in the breast cancer population. Is the breast cancer effect one of gender or is it the type of tumor?

Robert Diasio, MD: No, it is not the gender effect. As a matter of fact, we have taken a corresponding group of similar age colorectal cancer patients and head and neck cancer patients who were getting 5-FU and we do not find it. We do not understand what it is due to, but there is a definite shift. We have studied more than 500 breast cancer patients in our population and we have looked at other factors like menopausal status, adjuvant setting vs advanced disease, but we do not have any good explanation of any factor that is responsible for it. But there is a definite shift to the left of the normal distribution.

Dr. Haller: If DPD deficiency is seen in 5% to 6% of people, why do we see it so rarely in clinical practice? Is there an artificial cutoff point?

Dr. Diasio: I think that it is relative. We have seen it in a number of patients. The 6% figure I use for breast cancer is based on somewhere between 500 and 600 patients—all of the breast cancer patients who come to our clinic. Some of those patients were not getting 5-FU therapy. Others were. We continue to be impressed that though this is not a common problem, it is out there, and there are more patients who have this than I think we ever previously thought existed.

Dr. Haller: How many patients have you documented with lethal toxicity?

Dr. Diasio: Seven lethal patients. Those seven patients died following administration of chemotherapy, several of them after the first cycle of chemotherapy where they became septic. But we have a large percentage of patients who are heterozygotes and have only one defect. There are at least 13 different mutations that have been documented now.

The 5% to 6% DPD deficiency is specifically for breast cancer. With colorectal cancer, it is about 3% of the patients that we have found.

John Marshall, MD: Would it make sense to use a DPD inhibitor with capecitabine?

Dr. Diasio: It is something that has been proposed by several investigators and is being considered. I think at this point there is more focus really on the use of molecular markers to characterize the tumors. It is something that could be considered in the future. I think that a lot of the toxicities we feel are related to the catabolic pathway probably can be dealt with much in the way we deal with the Lokich infusional 5-FU regimen and that is, by turning down the amount of 5-FU either by pausing or reducing the dose.

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