The Role of Dihydropyrimidine Dehydrogenase (DPD) Modulation in 5-FU Pharmacology

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Over the past several years, the pyrimidine catabolic pathway and, in particular, the first enzymatic step involving dihydropyrimidine dehydrogenase (DPD) have been recognized as being critical in determining the ultimate...

Introduction

Dihydropyrimidine dehydrogenase (dihydouracil dehydrogenase, dihydrothymine dehydrogenase, uracil reductase, EC 1.3.1.2, DPD) is the initial rate-limiting enzyme in pyrimidine catabolism. DPD is important in the catabolism of not only the naturally occurring pyrimidines uracil and thymine, but also the widely used antimetabolite cancer chemotherapy agent 5-fluorouracil (5-FU).[1,2] DPD thus occupies an important position in the overall metabolism of 5-FU, converting over 85% of clinically administered 5-FU to 5-FU$H_2$, an inactive metabolite, in an enzymatic step that is effectively irreversible (Figure 1).[3] While anabolism is clearly critical in the conversion of 5-FU to the active nucleotides FdUMP, FUTP, and FdUTP (these metabolites can in turn inhibit cell replication through inhibition of thymidylate synthase, or through incorporation into RNA or DNA, respectively), catabolism controls the amount of 5-FU available for anabolism and thus occupies a critical position in the overall metabolism of 5-FU.

Assessing DPD Activity in the Clinical Setting

Because of the widespread use of fluoropyrimidines in oncology, it is particularly desirable to be able to assess DPD activity in the clinical setting. The ability to measure DPD in human tissue has further increased our appreciation of the importance of DPD for 5-FU pharmacology (see below). Currently there are three different methods used to measure DPD activity. Two of the methods are direct assays of DPD activity; one being an HPLC-radioassay for DPD activity and the second being an immunoblot assay of DPD protein.[3] A third method, useful mainly in the in vivo or clinical setting, is an indirect assessment of DPD activity. By quantitating the levels of uracil, the natural substrate for DPD that increases when DPD is inhibited, an estimate of remaining DPD activity can be made.[4]

For most clinical studies of DPD activity, peripheral blood mononuclear cells, isolated from heparinized blood on Ficoll-hypaque, can be used to monitor for DPD activity. Following preparation of cytosol, DPD activity can be assayed as follows: At a specific protein concentration of cytosol, incubation is carried out at 37°C in the presence of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) and radiolabeled 5-FU. Samples can then be removed at specified times (over 30 min) and analyzed by reversed phase HPLC using a radiodetector. DPD activity is then expressed as nmol of 5-FUH2 formed per min per mg of protein. This is described more fully elsewhere.[5]

The Critical Role of DPD in 5-FU Pharmacology

The importance of DPD to the clinical pharmacology of 5-FU has been shown in several recent studies that demonstrate how DPD can influence the pharmacokinetics, bioavailability, toxicity, and antitumor effectiveness of 5-FU.
DPD is known to have a circadian pattern in both animals and humans.[6-8] Studies in patients given 5-FU infusion by automated pumps have demonstrated that the circadian variation of tissue DPD level is accompanied by an inverse circadian pattern in plasma 5-FU concentrations. This has potential importance in the design of time-modified 5-FU infusions. Such regimens have been
suggested to have potential benefit in the treatment of certain human cancers.[9] DPD enzyme activity in normal tissues (peripheral blood mononuclear cells and liver) has also been shown to vary among individuals in a Gaussian pattern, with as much as a sixfold variation from the lowest to the highest values.[10,11] This wide variation in DPD activity is likely responsible for the wide variation in the $t_{1/2}$ observed in patients in population studies.[12] In addition to the variation of DPD activity observed in the normal population, it is now clear that an additional small percentage (< 5%) of the population has DPD activity significantly below the Gaussian distribution that characterizes most of the population.[13-15] These individuals are at significant risk if they develop cancer and are given 5-FU. This is a true pharmacogenetic syndrome with symptoms that are not recognized until patients are exposed to the drug.[16] Recently, variation in DPD activity has also been shown to be responsible for variable bioavailability observed following oral administration of 5-FU. The erratic bioavailability of 5-FU had not previously been understood, particularly because 5-FU is a relatively small molecule with a pKa that would predict excellent absorption and bioavailability. Experimental studies using DPD inhibitors have demonstrated in rodents 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, suggesting almost 100% bioavailability.[17] Tumors may also express a variable level of DPD activity.[18] This potentially may explain the observed varied tumor response to 5-FU.[19] Tumors with relatively low levels of DPD should predict sensitivity to 5-FU, while tumors with relatively high levels of DPD should predict resistance to 5-FU.

**Inhibition of DPD for Pharmacologic Gain**

The studies described above detailing the variability in DPD levels in both normal and tumor tissues provide an explanation for the observed variability in 5-FU pharmacology and at the same time suggest a potential target for chemotherapy. It thus becomes attractive for the oncologist to consider inhibiting DPD in order to eradicate the variability in 5-FU pharmacology. Inhibiting DPD in 5-FU-susceptible host tissue, such as GI mucosa and bone marrow, should make dosing from patient to patient less variable. Inhibition of DPD in tumor specimens is also attractive because it is likely that many tumors achieve resistance to 5-FU through an increase in DPD activity within the tumor resulting in increased degradation and thus less opportunity for anabolism of 5-FU.

Over the years there have been many attempts to synthesize effective inhibitors of DPD.[20] Unfortunately, many of these compounds have proven to be very toxic. In the past several years, new fluoropyrimidine drugs introduced into the clinic have shown that pharmacologic modulation of DPD can result in antitumor efficacy with tolerable toxicity.

**New Fluoropyrimidine Drugs Using Pharmacologic Modulation of DPD**

Four new fluoropyrimidine drugs using DPD modulators are currently being evaluated in clinical studies. These include UFT, eniluracil, S-1, and BOF-A2. These drugs differ in the type of DPD modulation as well as the relative degree of inhibition of DPD produced. All of these drugs achieve therapeutic gains from DPD modulation. Most impressive is the fact that DPD modulation permits oral administration of these drugs and results in less pharmacokinetic variability.

UFT was the first of these drugs to be synthesized (more than 20 years ago)[21] and is the one for which we have the most clinical experience. This novel 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 5-FU formed from tegafur will not be rapidly degraded and will remain present for a prolonged period (**Figure 2**). While not actual inhibition of DPD, the competition at the DPD level produces an effect similar to that accomplished with a true DPD inhibitor. In contrast to true DPD inhibitors and inactivators, the effect on DPD is more rapidly reversible. There are now extensive data from Japan[22] as well as Europe, South America, and the United States demonstrating that oral UFT administered either as a single agent or combined with leucovorin has antitumor activity in several tumor types (particularly breast and colon cancer) that is at least as good as that achieved with IV 5-FU. Furthermore, the toxicity profile has been shown to be tolerable, with the typical fluoropyrimidine toxicities (eg, diarrhea and nausea) seen at the maximal tolerated dose (MTD). Of note is the virtual absence of other toxicities, in particular hand-foot syndrome, neurologic toxicity, and cardiotoxicity.[23] Although not well understood, these toxicities have been thought to be possibly secondary to the formation of 5-FU catabolites. Because of modulation at the DPD level, 5-FU catabolites are less likely to form from UFT.
In Japan, there have been several attempts to develop this drug combination concept further. S-1 is a triple drug combination (Figure 3) consisting of the prodrug tegafur (same as in UFT) together with a true DPD inhibitor 3-cyano-2,6-dihydroxypyridine (CDHP) and potassium oxonate in a molar ratio of 1:0.4:1, respectively.[24] S-1 permits sustained 5-FU release from the 5-FU prodrug, with DPD modulation in this combination being more potent secondary to the effect of an actual DPD inhibitor. The third drug in this combination, potassium oxonate, was added theoretically to lessen the potential for typical 5-FU gastrointestinal toxicity (particularly diarrhea) seen with most fluoropyrimidine drugs. In preclinical studies, potassium oxonate was shown to selectively inhibit 5-FU phosphorylation by the enzyme orotate phosphoribosyltransferase, particularly in the gastrointestinal tract but not in tumors.[25] Results of preclinical studies have been encouraging, demonstrating excellent antitumor activity.[26] In clinical studies thus far, S-1 has been quite tolerable.[27,28]

BOF-A2 represents another attempt to develop an improved fluoropyrimidine drug. With this two-drug combination, the prodrug 1-ethoxymethyl fluorouracil (EM-FU) is combined with the DPD inhibitor 3-cyano-2,6-dihydroxypyridine (CNDP) in a 1:1 molar ratio.[29] EM-FU is relatively resistant to degradation and is metabolized to 5-FU by the liver microsomes. Preclinical studies have confirmed the antitumor activity of BOF-A2 in several animal models and have also demonstrated sustained 5-FU levels resulting from release of 5-FU from EM-FU. Clinical studies have been conducted in Japan, and more recently, limited studies have begun in the United States. It is too early to comment on the possible clinical effectiveness of this drug combination because of the limited patient data available. In US studies of BOF-A2 thus far, however, typical 5-FU toxicities were observed, with some patients experiencing more severe toxicity.[30] At present, the dose, schedule, and potential for combining this agent with other modulators (eg, leucovorin) are being evaluated.

Recently, eniluracil (ethynyluracil or GW776C85), an even more potent inhibitor of DPD, has been synthesized. This agent has been demonstrated to be a potent inhibitor of DPD.[31] Eniluracil is a pyrimidine with a structure similar to that of both uracil and 5-FU (Figure 4) that has been shown to rapidly and completely inactivate DPD (Figure 5). Animals exposed over prolonged periods to relatively low doses of eniluracil alone had no obvious toxicity.[32] Following the inhibition of DPD in these animals, a concomitant increase in plasma uracil has been observed.[33] Identification of an effective, nontoxic dose of eniluracil has permitted evaluation of this drug combined with various low doses of 5-FU. Results of pharmacokinetic studies in rodents demonstrated remarkably reproducible 5-FU pharmacokinetics.[34] Subsequent rodent studies showed that 5-FU could be administered orally in reproducible intra-animal and interanimal studies, with bioavailability being demonstrated to be almost 100%.[35]

The effectiveness of 5-FU and eniluracil in inhibiting tumor growth has been demonstrated in several animal models, with evidence of complete tumor regression in models in which only modest antitumor effect had previously been seen.[33] Initial phase I clinical studies with eniluracil examined administration of the drug alone orally for 7 days at doses of 0.74, 3.7, or 18.5 mg/m² to determine both the initial clinical pharmacologic characteristics and the toxicity profile. Pharmacokinetic evaluation demonstrated a t½ of 4.5 hours for each of these doses. No changes were noted in the pharmacokinetics of eniluracil with repeated doses compared with single doses.[34]

Of particular interest is the effect of these doses of eniluracil on DPD activity in the clinical setting. To assess DPD activity, peripheral blood mononuclear cells were examined using the methodology described above. Results demonstrated that DPD was rapidly and completely inactivated by eniluracil, and inhibition was maintained for more than 1 day at clinically used doses (Figure 6).[35] Currently, phase II studies are ongoing or planned to evaluate the effectiveness of the coadministration of 5-FU and eniluracil in several different malignancies. Improved efficacy of chemotherapy may result from eliminating 5-FU degradation by tumor DPD, making DPD inactivation appealing as a therapeutic goal. Studies are under way to evaluate whether eniluracil can produce this effect in clinical studies.[36] One concern, however, is the length of time DPD remains inhibited in normal host tissues, and clinical studies are currently planned to evaluate this potential toxicity.

**Conclusions**

In summary, DPD activity is a critical step in pyrimidine metabolism and is responsible for much of the variability in pharmacokinetics, oral bioavailability, toxicity, and efficacy following administration of 5-FU. Modulation of DPD activity through relative DPD inhibition should result in less variation in
5-FU pharmacokinetics and bioavailability and potentially may improve the drug’s therapeutic effectiveness both by making toxicity (after 5-FU dosing) more predictable and by overcoming the high levels of DPD activity in tumors where elevated DPD is a mechanism of tumor resistance. The recent availability of DPD modulators such as UFT, S-1, BOF-A2, and eniluracil offer the potential to decrease the variability due to DPD and produce an improved fluoropyrimidine therapeutic effect. The type of DPD modulation and relative degree of inhibition of DPD varies with each drug. These differences may in turn account for differences in efficacy and toxicity. Further investigation, including comparative studies, is needed to clarify these differences.

References:


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