Editorial

Dihydropyrimidine Dehydrogenase Activity: Prognostic Partner of 5-Fluorouracil?

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5-FU[2] is one of the most commonly prescribed anticancer agents having notable activity in the treatment of cancers arising from the breast, gastrointestinal tract, and head and neck. The metabolism, mechanisms of action and resistance, and pharmacokinetics of 5-FU have been extensively investigated since its synthesis over 4 decades ago. Despite a longstanding knowledge of the metabolic pathways governing the fate of 5-FU, it is only recently that cellular activities of particular metabolic steps critical in the metabolism of 5-FU have been used as a potential means for defining populations of patients at increased risk for the toxic side effects of the drug, as well as those who may derive the greatest clinical benefit from its use. DPD is the critical rate-limiting step in the catabolism of 5-FU and accounts for 80–90% of the drug's clearance. Thus, the level of this enzyme may be an important determinant in predicting toxicity associated with the use of 5-FU and potentially for predicting patients whose tumors are most likely to respond to treatment with 5-FU. In this issue of *Clinical Cancer Research*, Johnson *et al.* (1) describe severe multiorgan toxicity associated with the topical application of 5% 5-FU cream to the scalp of a 76-year-old male patient with basal cell carcinoma (1). This unusual and heretofore unreported example of severe toxicity associated with the topical use of 5-FU was shown by the authors to be the result of virtually undetectable DPD enzymatic activity in the PBMCs of the patient. Given the generally accepted safety of topical 5-FU use, this case represents a striking example of the severity of toxicities that may be associated with even minor exposure to 5-FU in patients with severe DPD deficiency. It has been estimated that ~3–5% of patients have PBMC DPD activity less than the 95% of the lower limit for the normal population (150 pmol/min/mg protein) and are considered to be at a high risk for the development of severe or life-threatening toxicities with the use of 5-FU (2–5). The risk of lethal toxicities is even greater for those patients with DPD activity ≤100 pmol/min/mg protein (≤99% of normal population).

Tuchman *et al.* (6) were the first to describe severe toxicity, including semi-coma associated with the use of 5-FU in a 27-year old woman undergoing adjuvant therapy for the treatment of breast cancer. Although these investigators did not directly measure DPD activity, the association of pyrimidinemia and pyrimidinuria in the patient, as well as in a sibling, suggested that the toxicity associated with 5-FU was the result of a genetic defect in the degradation of uracil and thymidine, most likely due to a deficiency of DPD. Subsequent investigations by *Diasio et al.* (7), in a second reported case of severe toxicity associated with the use of 5-FU, identified a complete lack of DPD enzymatic activity (7). Additional investigations in family members suggested an autosomal recessive pattern of inheritance. Although patients with severe or complete DPD deficiency have a high incidence of toxicity associated with the use of 5-FU, there does not seem to be a significant correlation between DPD levels within the normal range and 5-FU-associated toxicity (8). Thus, the potential role for DPD quantitation in normal tissues (PBMCs) may be limited to the identification of patients with DPD levels 2 or 3 SDs lower than the population mean and are, thus, at the highest risk for toxicity, particularly neurotoxicity, associated with the use of 5-FU. It is important to note that although the greatest site for 5-FU catabolism is in the liver, the PBMC DPD levels have been shown to closely correlate with DPD activity in normal liver, thus supporting the use of PBMCs as surrogate for total body DPD activity (9). However, recent investigations have identified monocytes as containing 3-fold more DPD activity than lymphocytes, thus the relative ratios of various mononuclear cell fractions needs to be accounted for particularly in those patients whose levels of DPD approach the 95th percentile (10). For those patients who develop severe neurological toxicity associated with the use of 5-FU in the setting of DPD deficiency, infusional thymidine may be considered a potential rescue agent. A report by Takimoto and colleagues demonstrated the value of such an approach in a patient who developed severe encephalopathy associated with the use of 5-FU and who was subsequently found to be severely DPD deficient (11).

In addition to its role as a predictor of toxicity associated with 5-FU use, DPD levels may also aid in the prognostication of patient populations whose tumors are likely to respond to 5-FU. Low levels of DPD activity in normal tissues may predict for a greater fluorouracil exposure and, thus, the possibility of enhanced tumor response. Perhaps more critical are intratumoral levels of DPD that regulate intracellular 5-FU levels and, thus, may be an important determinant of clinical outcome. Preclinical work using cancer cell lines found DPD levels to be, at best, only poorly correlated with 5-FU responsiveness (12, 13). Nonetheless, several groups have investigated the predictive value of DPD as an indicator of sensitivity to 5-FU in patients. These results, albeit in relative small numbers of patients, suggest that low levels of intratumoral DPD expression are associated with 5-FU responsiveness (14–16). Given the ease with which PBMCs may be obtained, it has been suggested that this tissue may serve as a surrogate for DPD activity in tumor tissue. However, a recent investigation of colorectal tumors taken from 57 patients found only a poor correlation of DPD levels in tumor tissue with that in the PBMCs, thus supporting the need for

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[2] The abbreviations used are: 5-FU, 5-fluorouracil; DPD, dihydropyrimidine dehydrogenase; PBMC, peripheral blood mononuclear cell.

tumor tissue measurements of DPD in the assessment of DPD as a potential predictor of 5-FU responsiveness (17). Clearly, the value of DPD as a predictor of 5-FU responsiveness will need to be explored in larger numbers of patients in a prospective fashion before definitive conclusions may be reached regarding its use.

In the present study, the authors identified the molecular defect responsible for the complete lack of DPD enzymatic activity, namely, a G to A mutation in a 5′ splicing recognition sequence, which results in deletion of all 165 bp of exon 14. This particular mutation, as well as a variety of other point mutations and deletions of the *DPD* gene, were identified previously (18–22). Perhaps the most complete cataloging of mutations that result in complete deficiency of DPD has been undertaken by Van Kuilenburg *et al.* (18), wherein 17 families representing 22 patients with complete deficiency of DPD were studied (18). Although seven different mutations were identified, more than half of the patients demonstrated the identical G to A point mutation described in the present study, suggesting that this is probably the most common mutation identified to date that is associated with complete absence of DPD activity. Despite the presence of various clinical abnormalities in the patients with absence of DPD activity, no definitive genotype-phenotype associations have been identified.

Ubiquitination is becoming recognized as a common mechanism regulating a host of cellular processes with well over 50 ubiquitination substrates having been identified. In general, ubiquitination targets proteins to the 26S proteasome wherein the polyubiquitinated proteins are degraded. It has been shown that not all ubiquitinated proteins are targeted for degradation, and it has been postulated that this posttranslational modification may have other critical cellular functions. It is interesting that the authors of the present study describe ubiquitination of the mutant DPD protein. Several possible explanations exist for this observation. The ubiquitination process may be part of the normal cellular physiology that regulates the level of DPD. Implicit is the notion that dysregulation of this process may represent a means for overexpression and, therefore, tumor resistance to 5-FU. If ubiquitination was part of normal cellular physiology, one would need to postulate why ubiquitin is observed on the mutant DPD protein, but not wild type. The process of ubiquitination has also been described as a cellular mechanism designed to scavenge and degrade mutant or misfolded proteins (23, 24). This latter possibility represents an alternative explanation that would also account for the lack of observed ubiquitination of the wild-type enzyme, but may not constitute a mechanism for regulating enzyme activity, assuming the mutant forms are already catalytically inactive. Whether ubiquitination of DPD is part of the normal cellular physiology or represents the cells attempt to simply rid itself of aberrant molecules will await further investigations. In either case, the role of ubiquitination as a regulator of DPD represents an important area for future investigations given the relative importance of DPD as a potential predictor of both response and toxicity associated with the use of 5-FU.

Given the potential value of DPD as a determinant of both tumor responsiveness and toxicity associated with 5-FU, recent attempts have focussed on modulating the activity of 5-FU through the inhibition of DPD. Several agents are undergoing clinical testing and include UFT, which contains the 5-FU prodrug ftorafur in a 1:4 molar ratio with uracil, a competitive inhibitor of DPD; eniluracil (ethynyluracil, GW776C85), a direct inhibitor of DPD; and S-1, which contains a direct inhibitor of DPD (5-cloro-2,4-dihydroxypyridine). The hope is that these agents will prolong the intracellular half-life of 5-FU through inhibition of intratumoral DPD, but these compounds also inhibit DPD found in the normal tissues responsible for clearance of the drug, thus markedly prolonging the total body exposure to 5-FU with associated increased toxicity. A critical issue will be whether the use of these inhibitors can enhance the therapeutic index of 5-FU through relatively selective tumor *versus* normal tissue DPD inhibition. However, even in the absence of a selective advantage, the use of DPD inhibitors has provided a means for developing oral 5-FU-based regimens, an important advance in convenience and quality of life for patients requiring 5-FU therapy. One caveat is that other agents used for noncancer indications, such as the antiviral sorivudine, can have important drug-drug interactions through the inhibition of DPD. This particular antiviral agent led to the death of 16 patients in Japan who were being treated with the antiviral medication while undergoing therapy with 5-FU (25).

The report by Johnson *et al.* (1) illustrates the critical role of DPD in governing 5-FU-associated toxicity in patients with severe enzyme deficiency. The role of DPD as a predictor of toxicity for patients with levels within the normal range and as a predictor of tumor response awaits further clinical investigations. The prediction of tumor response based on the make-up of molecular response determinants in a given individual's tumor is a critically important area for future investigation and holds enormous promise for advancing the field of cancer therapeutics.

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