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Review

Can dihydropyrimidine dehydrogenase impact 5-fluorouracil-based treatment?

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Abstract

More than 80% of an administered 5-fluorouracil (5-FU) dose is degraded by dihydropyrimidine dehydrogenase (DPD), making it an important regulator of this commonly used anticancer agent. The high variation in population DPD activity, association with 5-FU activity, and development of DPD inhibitors have all contributed to the current focus on this enzyme. This review details the impact of DPD on 5-FU pharmacology, catalogues recent information on DPD mutations, evaluates the case for tumour DPD as a source of 5-FU resistance and introduces the clinical case for DPD inhibitors as a mechanism for the use of oral fluoropyrimidine therapies.

Keywords: 5-Fluorouracil; Dihydropyrimidine dehydrogenase; Oral chemotherapy

1. Introduction

The domain of fluoropyrimidines is currently expanding quickly with the clinical development of oral 5-fluorouracil (5-FU) prodrugs such as UFT, S1, capecitabine or oral 5-FU combined with 5-ethynyluracil [1]. The use of oral fluoropyrimidines underlines the importance of the enzyme dihydropyrimidine dehydrogenase (DPD) which not only controls the catabolic elimination of 5-FU, but also limits its oral absorption [2]. DPD inhibition has become a major goal of the strategy for the development of oral fluoropyrimidines like UFT, S1 or 5-FU–ethynyluracil. For instance, UFT preparation incorporates uracil which is a competitive inhibitor of DPD and S1 includes 5-chloro-2,4- dihydroxypyridine which is also a stronger competitive inhibitor of DPD. 5-Ethynyluracil makes an irreversible complex with DPD.

DPD has highest activity in liver and mononuclear cells, but is also found in most human tissues. DPD also demonstrates variable activity in human tumours and variation in tumoral DPD may potentially influence the efficacy of 5-FU [3]. The aim of the present review was to summarise the current knowledge on DPD including pharmacokinetic–pharmacodynamic aspects, molecular considerations for the *DPYD* gene and the clinical development of DPD inhibitors. Our aim was to try to bring concrete answers to current questions that one may ask concerning the impact of DPD on 5-FU-based chemotherapy.

2. DPD and 5-fluorouracil clearance

5-FU has been in clinical use for over 40 years and is the third most commonly prescribed chemotherapy agent [2]. 5-FU is used as a single agent to treat colorectal cancer and is a significant component of combination therapy for breast, head/neck and upper gastro-intestinal malignancies. More than 80% of an administered dose of 5-FU is eliminated by catabolism through DPD, the rate-limiting enzyme (Fig. 1) [2]. DPD activity is found in most tissues, exhibiting the highest activity in the liver. However, peripheral blood mononuclear cells (PBMC) are used for clinical monitoring of DPD activity, as these cells are more accessible than

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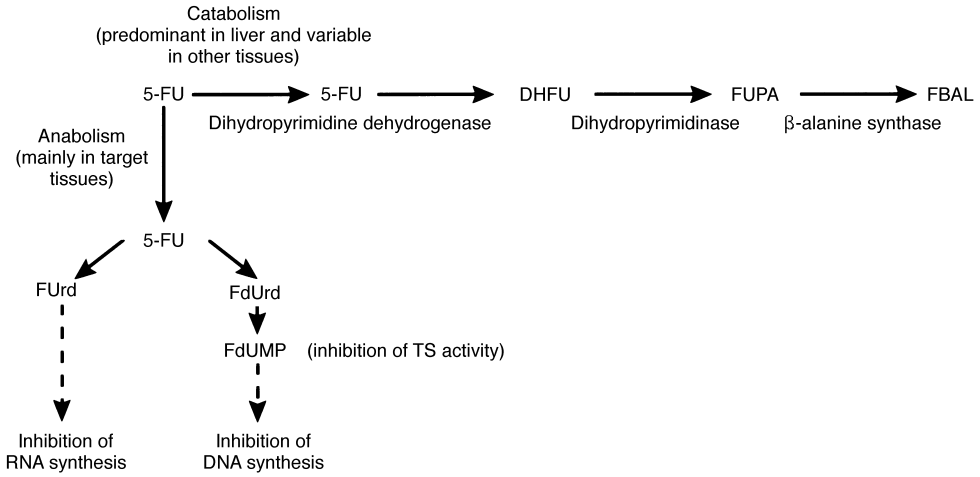

Fig. 1. Pathways of 5-FU metabolism. DHFU, 5′, 6′-dihydrofluorouracil; FUPA-α-fluoro-β, ureidopropionic acid; FBAL, 5-fluoro-β-alanine; FUrd, fluorouridine; FdUrd, fluorodeoxyuridine; FdUMP, fluorodeoxyuridine monophosphate; TS, thymidylate synthase.

hepatic tissue. A significant, but weak ($r^2 = 0.32$), correlation between PBMC and liver DPD activity has been observed [4]. The relationship between PBMC DPD activity and 5-FU systemic clearance has been evaluated by Fleming and associates [5]. A significant linear correlation has been observed between PBMC–DPD activity and 5-FU clearance [5, 6]. However, this relationship is relatively weak ($r^2 = 0.10$) and even though related to 5-FU clearance, simply determining PBMC–DPD is not sufficient to accurately predict 5-FU clearance. Recent NONMEN population pharmacokinetic analysis identified patient co-variables which could influence interpatient variability in 5-FU clearance [7]. 5-FU clearance was significantly reduced by increased age, high serum alkaline phosphatase, length of infusion and low PBMC–DPD. However, a relatively high error was found in the estimate between observed and predicted 5-FU clearance and thus this multifactorial approach including PBMC–DPD did not allow faithful 5-FU dose adaptation prior to treatment. In addition, DPD activity may vary from one cycle to the other without any evidence of a trend for an increase or decrease during the treatment course [5]. There is also now some evidence that autoregulation of 5-FU metabolism takes place, in that inhibition of DPD activity was observed after 5-FU administration in both colorectal cancer patients and an animal model [8]. Maximum inhibition occurred 48 h after 5-FU administration although the molecular basis for the alteration is not known. Thus, a PBMC–DPD-based 5-FU dose adaptation strategy is not justified in our opinion.

3. DPD deficiency

In order to evaluate the incidence of complete or partial DPD deficiency, several prospective studies have been conducted. Lu and associates [9] were the first to provide such data and demonstrated a Gaussian distribution for PBMC–DPD in 124 healthy subjects. We recently performed prospective studies on 185 unselected cancer patients and 75 colorectal cancer patients [6, 10]. In these populations, DPD activity also showed a unimodal distribution and no subject with complete DPD deficiency was identified in these studies. Multifactorial analysis of variance showed that neither liver function tests (biological evaluation) nor age influenced DPD activity, but DPD activity was, on average, 15% lower in women as compared with men ($P = 0.03$) [6]. Interestingly, this 15% difference in DPD activity is of the same order as the difference observed in 5-FU clearance between men and women [11]. In the study by Lu and colleagues, DPD activity was not influenced by sex [9]. The discrepancy in the effect of gender on DPD activity between these studies could be explained by the difference in the age range covered, with influences from the hormonal status (premenopausal women were the majority in the Lu study versus postmenopausal women in the majority studied by Etienne and colleagues [6]). However, this hypothesis could not be confirmed from the limited set of women studied ($n = 33$), since no difference in DPD activity was demonstrated between pre- and postmenopausal women. In total, from these studies [6, 9, 10], it is clear that complete DPD deficiency is a very rare event. However, if we consider the PBMC–DPD value of 100 pmol/min/mg protein as the upper threshold indicative of an increased risk for developing 5-FU-related toxicity [3], one can estimate that approximately 3% of an unselected group of cancer patients are located below this threshold value [6]. DPD-associated morbidity, and in some cases mortality, among patients who often do not have detectable disease (adjuvant therapy) has great personal and economic implications. It follows that, in our opinion, the practical interest to determine DPD before 5-FU treatment must be carefully weighed in terms of cost–benefit

balance. Current methods, requiring PBMC isolation and high performance liquid chromatography (HPLC) analysis, are not applicable for general screening. However, development of new, less labour-intensive assays may prove more amenable to prospective use in cancer units.

4. Molecular studies

The molecular basis for DPD deficiency has been identified in several patients with severe 5-FU toxicity [10]. A G→A mutation in the exon 14 splice donor site has been identified which leads to skipping of the 165 bp exon [10, 12]. Analysis of this mutation among Caucasian colorectal patients, as well as healthy volunteers, found an incidence of 1 in 270, suggesting that there are more mutations to be identified to explain the molecular basis for DPD deficiency [10, 13]. Although the majority of mutations identified to date do result in a change in the encoded amino acids, these mutations are not specifically found in patients with low DPD activity (Fig. 2; [13]). For example, an A→G change at nucleotide 1627 results in production of a valine, rather than the wild-type isoleucine. However, this polymorphism occurs in 28% of all alleles and is not associated with low PBMC–DPD activity [13]. A similar pattern is observed for a G→A change at nucleotide 2194, which is found in 5% of the general population. Although this change alters amino acid 732 from valine to isoleucine, it has been identified in patients with both high and low DPD activity [13]. Therefore, these mutations are probably common polymorphisms which do not have direct relevance to the identification of patients at risk for 5-FU toxicity. Only the G→A mutation in the exon 14 splice site is a confirmed molecular basis for 5-FU toxicity due to low DPD activity [12]. We conclude that DPD deficiency phenotyping cannot yet be substituted by DPD genotype analysis.

5. Circadian rhythm

The existence of a circadian rhythm for DPD activity has been suggested from both human and animal investigations [14]. Harris and associates [15] measured PBMC–DPD and 5-FU plasma concentrations in cancer patients receiving 5-FU by protracted continuous infusion. A circadian rhythm was observed in 5-FU plasma concentration with a peak observed at 11 am and a trough at 11 pm on average. This inverse relationship observed between the circadian profile of 5-FU plasma concentration and PBMC–DPD activity confirmed the link between DPD activity and 5-FU pharmacokinetics. However, several studies have described a wide interindividual variation in peaks and troughs in DPD activity and have suggested that the circadian cycle does not occur over 24 h in all subjects [16, 17]. Clearly, we feel that the fact that each subject exhibits his/her own profile of circadian rhythm for DPD limits a priori the significance of a given DPD determination for predicting 5-FU pharmacokinetics.

6. DPD in tumours

In addition to its role in 5-FU toxicity, DPD activity may be a potential factor for controlling 5-FU responsiveness at the tumoral level. A high level of tumour DPD would metabolise 5-FU to inactive products before cytotoxic nucleotides can be formed. The potential role of DPD for influencing 5-FU activity also concerns new 5-FU prodrugs like capecitabine or UFT, where 5-FU is metabolically produced at the target site. DPD activity *in vitro* in tumour cells was significantly related to 5-FU sensitivity [18]; the lower the enzymatic activity, the greater the cytotoxicity. Recent studies in human cancer xenografts demonstrated that the efficacy of capecitabine correlated very well with the ratio of thymidine phosphorylase/DPD [19]. The role of

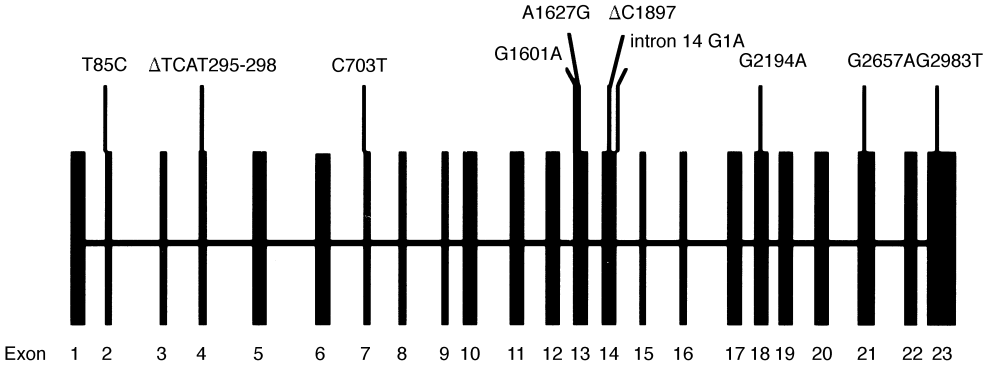

Fig. 2. The molecular structure of *DPYD*, the gene encoding for dihydropyrimidine dehydrogenase, with the nucleotides of known mutations highlighted. The wild-type nucleotide is listed prior to the cDNA sequence location, followed by the variant nucleotide. A deletion of nucleotides is indicated by Δ, followed by the nucleotide location. The nucleotides are numbered from the initiation start site [35].

tumoral DPD activity was recently evaluated in the clinical setting. For head and neck cancer patients, DPD activity was detectable in all tumour samples (median tumoral DPD activity was 60, range 13–193 pmol/min/mg protein) [20]. Tumoral DPD activity was not influenced by tumour staging. The patients with a complete response to 5-FU-based induction chemotherapy, exhibited lower tumoral DPD activities as compared with partial or non-responding patients [20]. In an attempt to reduce the variability due to confounding factors, including a possible circadian variability for DPD activity, we tested a normalised DPD value defined as the tumoral:adjacent non-tumoral ratio of DPD activity. Interestingly, the distribution of normalised DPD reveals that complete responders exhibit a significantly lower normalised DPD than partial or non-responding patients ($P = 0.03$) [20]. The tumour:normal tissue ratio is not the same for all tumour-types. A study of 63 colorectal tumours found a median tumour:normal ratio of 0.76 [21]. Although a subset of patients did have up to three times higher tumour DPD, the majority of patients had highest DPD in adjacent normal tissue. This may help explain the activity and mild toxicity of 5-FU in colorectal cancer, in that the tumour metabolic profile favours the formation of cytotoxic nucleotides while normal tissue will have greater inactivation. Although resistance to 5-FU is multifactorial, we consider that tumoral DPD activity may be a determining factor for 5-FU-responsiveness in a subset of cancer patients. In addition, these data provide further pharmacological rationale for the use of DPD-specific inhibitors.

7. DPD inhibitors

Several agents which affect DPD are under development (Table 1). 5-Ethynyluracil is unique in that it is a DPD inactivator, rather than an inhibitor, as it forms a suicide substrate for the enzyme [22]. This means that DPD activity will be lower for a prolonged period of time and it has been recommended that standard dose 5-FU not be administered for at least 2 months after the cessation of 5-ethynyluracil therapy, to avoid untoward effects. The pharmacokinetics of 5-FU are dramatically modified when 5-FU is combined with 5-ethynyluracil (GW776; eniluracil) [23–25]. The maximum tolerated dose for 5-FU in combination with 5-ethynyluracil was 25 mg/m^2/day for 5 days or 1 mg/m^2/day for 28 days, up to 50 times lower than that found for intravenous (i.v.) 5-FU alone [24, 25]. With 5-ethynyluracil pretreatment, the bioavailability of 5-FU becomes complete and renal clearance becomes predominant with a high correlation between 5-FU clearance and creatinine clearance [24]. This suggests that dosage reductions will need to be made in patients with poor renal function who are to receive the combination of 5-ethynyluracil and 5-FU. However, there are no published studies of this combination in patients with altered renal function.

Competitive inhibitors of DPD activity are also part of the new products UFT and S1 [26]. UFT contains uracil and tegafur in a 4:1 ratio and S1 includes 5-chloro-2, 4-dihydropyridine (CDHP) in combination with tegafur and potassium oxonate [1]. Both uracil and CDHP directly compete with 5-FU for the uracil binding site on the DPD protein, allowing more 5-FU to be available for the activation pathways. The inhibitory effect of uracil and CDHP on DPD is rapidly reversible. Therefore, more frequent dosing is required, but the short-lived nature of the inhibition may allow more flexibility in the use of standard dose 5-FU.

Preliminary reports of phase II/III trials for 5-ethynyluracil plus oral 5-FU, oral UFT and oral S1, as single agents, with leucovorin, or in combination with other cytotoxics, have demonstrated antitumour activity. 5-ethynyluracil/5-FU had a 52% objective response (OR) rate as first-line therapy for advanced breast cancer and demonstrated activity in anthracycline/paclitaxel refractory disease [27, 28]. A 26% OR rate was observed in chemotherapy naïve head/neck patients, but data are not yet available from the phase III studies in colorectal cancer or when combined with leucovorin or cytotoxic agents [29]. Similar data are emerging for UFT. A 26% OR rate was seen with UFT plus leucovorin in locally advanced and metastatic head/neck cancer [30]. The combination of UFT/leucovorin and paclitaxel was clearly active as second-line therapy for metastatic

Table 1
DPD-interacting agents under clinical evaluation to modulate fluoropyrimidine therapy

Compound	Chemical name	Effect on DPD	Cytotoxic component
Eniluracil (Glaxo-Wellcome)	5-ethynyluracil	Inactivator	5-FU
UFT (Orzel®, Bristol-Myers Squibb, contains UFT plus leucovorin)	Uracil + tegafur	Inhibitor	Tegafur
S1 (Bristol-Myers Squibb)	5-Chloro-2, 4-dihydropyridine + tegafur + potassium oxonate	Inhibitor	Tegafur

breast cancer (OR 31%) [31]. In preliminary results from two randomised trials of UFT/leucovorin versus i.v. 5-FU in metastatic colorectal cancer, the regimens were equally effective (OR rate 12% versus 15% and 11% versus 9%, respectively) [32, 33]. However, the UFT/leucovorin arm had fewer episodes of febrile neutropenia and infection. S1 is also an active oral therapy, with a 28% OR rate in head/neck cancer [34]. Phase III studies are maturing to define the efficacy of S1 in common solid tumours. A striking feature of the DPD inhibitor studies is the low (or absent) incidence of hand/foot syndrome. Diarrhoea and haematological toxicity appears to be what is expected from conventional 5-FU, but mucositis/stomatitis is also less than anticipated. These agents have the potential to dramatically change the way 5-FU is used in the future. Although current DPD inhibitors can alter the pharmacokinetics of 5-FU and inhibit DPD at the tumoral level, thus suppressing a possible cause of drug resistance, the inhibition will occur in both normal and tumour tissues. Therefore, the next step forward could include development of tumour-specific DPD inhibitors which could improve, at least theoretically, the therapeutic index of 5-FU or 5-FU prodrugs.

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