UFT: Mechanism of Drug Action

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The mechanism of action of fluorouracil (5-FU) and the oral fluoropyrimidines and the importance of biochemical modulation and inhibition of dihydropyrimidine dehydrogenase for oral application of the prodrugs of 5-FU are discussed.

**Introduction**

Fluoropyrimidines are among the most widely used antineoplastic agents with activity against breast, gastrointestinal, and head and neck malignancies. Since their synthesis by Heidelberger[1] during the 1950s, fluoropyrimidines have undergone extensive preclinical and clinical evaluation. The antineoplastic activity of fluorouracil (5-FU) is improved by biochemical modulation[2,3] and when administered as a continuous infusion.[4] Although the oral absorption of 5-FU is erratic and results in unpredictable and variable plasma levels in patients,[5] 5-FU prodrugs are pharmacologically more predictable. These compounds are being evaluated clinically and will likely become important drugs in the care of patients.

**Prodrugs of 5-FU**

Fluorouracil and its analogs tegafur and capecitabine (Xeloda) are shown in Figure 1. After administration, 5-FU is rapidly catabolyzed by the rate-limiting enzyme dihydropyrimidine dehydrogenase (DPD), which has its highest level of activity in the liver. This accounts for the high clearance of the drug, with only 5% to 10% entering anabolism into active compounds.[6,7] In addition, approximately 2% to 4% of the population is DPD-deficient due to genetic polymorphism in DPD activity.[8]

The oral fluoropyrimidines may be divided into two categories: those administered in combination with a DPD inhibitor and those administered without. Inhibition of DPD activity may be competitive with 5-FU, as in the case of uracil and 5-chloro-dihydropyridine; 5-ethynyluracil acts as a suicide inhibitor by inactivating DPD.

Capecitabine, a non-DPD 5-FU prodrug, becomes cytotoxic only after conversion to 5-FU. Following oral administration, capecitabine is metabolized in the liver by carboxyl esterase into 5-deoxy-5-fluorocytidine and by cytidine deaminase into 5´-deoxy-5-fluorouridine (Figure 2). The last step of conversion occurs with pyrimidine phosphorylase, which is shown to be at a higher concentration in the tumor than in normal tissue, with the potential advantage of greater tumor selectivity.[9]

Tegafur is a prodrug that slowly metabolizes into 5-FU through the activity of hepatic cytochrome P450 and systemic soluble enzymes;[10,11] 5-FU is the end product of this reaction. In order to increase the concentration of 5-FU, and prevent degradation of 5-FU by DPD, uracil is added at a molar concentration of 1:4 (5-FU:uracil). In preclinical models,[12] this molar combination has been identified as the most efficient.

S1 is a combination of tegafur and two modulators that prevent degradation. Tegafur, potassium oxonate, and 5-chloro-2,4-dihydropyridine (CDHP), are combined in a molar ratio of 1:0.4:1.[13,14] CDHP is a competitive, reversible DPD inhibitor that prolongs the half-life of 5-FU. Oxonic acid is a pyrimidine phosphoribosyltransferase inhibitor that is added to prevent the phosphorylation of 5-FU in the digestive tract with the aim of reducing 5-FU-related gastrointestinal toxicity.[15] The intravenous (IV) formulation of 5-FU may be given as an oral drug when it is combined with the oral DPD inhibitor ethynyluracil. Pretreatment with ethynyluracil results in 100% oral bioavailability and extends the half-life of 5-FU from less than 10 minutes to over 100 minutes.[16] In these circumstances, 5-FU has linear pharmacokinetics and clearance is primarily renal. Complete DPD inhibition might last for several weeks after ethynyluracil is discontinued,[17,18] thus preventing further conventional dosing of 5-FU for several weeks or even months.
5-FU Mechanism of Action

All 5-FU prodrugs and 5-FU combined with DPD inhibitors exert their antineoplastic activity in a similar manner. The biochemical modulation of 5-FU is reviewed in Figure 3. 5-FU may act via thymidylate synthase (TS) inhibition through its active anabolites such as fluorodeoxyuridine monophosphate (FdUMP). This reaction is facilitated by the formation of a ternary complex consisting of FdUMP, TS, and 5,10-methylene tetrahydrofolate (5,10-MTHF). The intracellular concentration of 5,10-MTHF can be increased through the addition of other reduced folates such as leucovorin, thus promoting a greater stability of the ternary complex. Thymidylate synthase catalyzes the conversion of dUMP to TMP, which is a precursor of TTP, one of the four deoxyribonucleotides required for DNA synthesis. Also, incorporation of the anabolites of 5-FU into RNA and DNA contributes to the antineoplastic activity of 5-FU.[19,20] In preclinical models, interferon demonstrated the ability to enhance the cytotoxicity of 5-FU by several potential mechanisms, including affecting thymidine kinase activity, depleting intracellular thymidine concentration,[21] and most likely, enhancing DNA damage.[22,23]

Pretreatment Strategies

Pretreatment with methotrexate results in an increased intracellular concentration of phosphoribosyl pyrophosphate (PRPP), the cofactor for conversion of 5-FU into fluorouridine monophosphate (FUMP) and, subsequently, into 5-fluorouridine 5´-triphosphate (FUTP).[24,25] The administration of high-dose methotrexate is usually accompanied by leucovorin rescue, which can lead to a dual effect. Leucovorin will not only protect against methotrexate-induced toxicity, but can also interfere with methotrexate uptake, thereby abolishing its effect. In addition, administration of leucovorin will result in direct modulation of 5-FU.[26,27] The net effect seems to be a leucovorin-mediated modulation. Therefore, methotrexate was substituted with trimetrexate, which, due to its lipophilicity, does not require the reduced folate carrier. This combination was very effective in vitro, and is currently being evaluated in the clinic. Phosphonacetyl-L-aspartate (PALA) is an inhibitor of aspartate transcarbamylase,[28] an important enzyme in the de novo synthesis of uridine and cytidine nucleotides. Pretreatment with PALA can yield a higher incorporation of 5-FU nucleotides into cellular RNA,[29] and a depletion of dUMP, leading to enhanced inhibition of TS. After transport of preformed extracellular thymidine into cells, it is anabolized by thymidine kinase to thymidine triphosphate (dTTP) and replete dTTP pools, thereby bypassing the 5-FU-induced depletion of dTTP.[30] Dipyridamole interferes with the cellular uptake of nucleosides. In tumor cells, in which the inhibition of TS is critical, dipyridamole results in depletion of dTTP pools by blocking the facilitated transport of exogenous thymidine. Unfortunately, in vivo application of dipyridamole is hampered by its high protein binding, thus preventing a dipyridamole-induced inhibition of nucleoside transport. All of these biochemical strategies have been extensively studied in clinical trials.[31,32] Methotrexate, PALA, alpha-interferon, DP have all been investigated as biochemical modulators, but the biochemical modulation by leucovorin appears to be most efficient resulting in a doubling of the response rate.[3]

The rationale for combining UFT (uracil and tegafur) plus oral leucovorin (a combination being developed under the trade name Orzel) derives from extensive evaluation of leucovorin modulation of 5-FU. As outlined in Figure 4, UFT plus leucovorin produces a double 5-FU modulation. A 1:4 molar combination of the 5-FU prodrug tegafur with uracil as a DPD inhibitor was developed two decades ago in Asia. Preclinical studies demonstrated that tegafur plus uracil resulted in higher tumor/blood ratios and greater tumor activity.[33,34] In addition, tegafur plus uracil was associated with lower central nervous system and gastrointestinal toxicity compared to tegafur alone.[35] As uracil is added to tegafur, the half-life of 5-FU is prolonged due to the inhibited ability of DPD to degrade 5-FU into alpha-fluoro-beta-alanine.

5-FU Resistance and Downstream Events

Only about 20% of patients with advanced colorectal cancer respond to the combination of 5-FU and leucovorin via bolus administration, leaving approximately 80% who fail to respond. Additionally, responses usually last for a short time, with most patients failing to respond later due to possible secondary resistance. Resistance to fluoropyrimidines is multifactorial and may be related to their antipyrimidine or antifolate properties (Table 1).[36] A general form of fluoropyrimidine resistance is the induction of TS activity at the initiation of 5-FU treatment (Figure 5).[37-40] This phenomenon
has been observed in a number of model systems both in vivo and in vitro. Using an in vivo murine colon tumor model, Van der Wilt and coworkers[39,41] observed the development of resistance following weekly bolus injections and after continuous administration of 5-FU for 10 to 21 days in tumors that were initially sensitive to such treatment. This resistance was associated with a rapid increase in TS levels, while plasma 5-FU levels under these conditions were comparable to the levels that had been achieved during the first days of infusion. Interestingly, pretreatment with leucovorin depressed this 5-FU-induced TS increase after the bolus injections. Sobrero and coworkers observed that low-dose, continuous exposure of 5-FU almost immediately resulted in resistant clones of the HCT-8 colon tumor cell line, whereas short-term exposure to 5-FU required a longer period to induce resistance.[42] Cells resistant to intermittent short exposure were sensitive to continuous exposure, but cells resistant to continuous exposure were also resistant to short-term exposure. The observation that the increase in TS was less pronounced if cells were pretreated with leucovorin[39] may serve as an explanation for the higher response rate observed with 5-FU therapy when modulated by leucovorin. Other investigators observed a similar increase in TS levels after treatment with 5-FU in a tumor cell model.[43,44] In this experiment, gamma-interferon was able to significantly reduce TS induction.

**Deregulation of Thymidylate Synthase Protein Synthesis**

The increase in TS levels is most likely explained by a deregulation of the normal TS protein synthesis. Under certain physiologic conditions, TS protein synthesis is related to the cell cycle, with high activity associated with the S-phase.[45] The translation of TS mRNA appears to be controlled by its end product, the TS protein, in an autoregulatory manner. However, when TS is bound to a ternary complex, the protein can no longer regulate its synthesis, leading to the observed increase. Expression of TS appears to be managed by a translational regulatory process during the cell cycle as well as in response to cytotoxic agents.

The capacity of TS as an RNA-binding protein was demonstrated by Chu and coworkers.[46] Nine different cellular RNAs were shown to form a ribonucleoprotein complex with TS in intact colon cancer H630 cells. Several of these isolated RNA sequences display a high degree of homology to those encoding the human p53 tumor suppressor protein, the c-myc and l-myc family of transcription factors, and the human zinc finger 8 transcription factor. p53 and c-myc encode for nuclear phospho-proteins that play central roles in the regulation of cell cycle progression, DNA synthesis, and apoptosis.[47,48]

**Apoptosis Regulation**

Interesting insights into the more downstream events of 5-FU antineoplastic activity have been reported by Houghton and coworkers,[49] who used a TS-negative (TS-)...
Recently, inhibition of angiogenesis by UFT and its metabolites has been reported.[55] A murine renal cell carcinoma (RENCA) cell line was studied as an angiogenesis model using a dorsal air skin assay. Blood vessels are formed by an angiogenic factor released from these malignant tumor cells. UFT showed a strong angiogenesis inhibitory effect whereas 5-FU and doxifluridine did not. The angiogenesis was apparently mediated by the degradation products of tegafur, gamma-hydroxybutyric acid, and gamma-butyrolactone. The inhibitory effect was amplified even when these compounds were administered by continuous infusion. While the clinical relevance of this observation remains to be determined, it is important to note that, in addition to its cytotoxic effects, UFT may have the potential to inhibit angiogenesis. This might be of special interest in the adjuvant setting.

**Conclusions**

Oral fluoropyrimidines are interesting compounds in the treatment of patients with solid tumors. As they are metabolized into 5-FU, their mechanism of action is basically similar to that reported with IV administration of 5-FU. Biochemical modulation also may apply to oral fluoropyrimidines, such as the combination of UFT plus oral leucovorin. Evidence is emerging that further downstream effects of fluoropyrimidines are related to proteins involved in the regulation of apoptosis. The effect of UFT on angiogenesis deserves further exploration.

**References:**


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