Clinical Implications of 5-FU Modulation

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In recent years, due to the advent of sensitive instrumentation and methodologies, it has been possible to identify parameters that predict the quality of response of individual patients to treatments for specific selected diseases.

Introduction

Fluorouracil (5-FU) has been utilized extensively as a single agent or in combination with other agents in the clinical management of gastrointestinal malignancies and carcinomas of the breast and ovary, and squamous carcinomas of the head and neck.[1,2] Administered as a single agent, 5-FU is generally considered the standard therapy for patients with adenocarcinoma of the colon; however, the drug produces a response rate of < 20% in this disease,[1] with a median survival of < 9 months. Because of the wide spectrum of marginal antitumor activity exhibited by 5-FU, numerous investigators are trying to enhance the antitumor activity and therapeutic selectivity of the compound by combining it with other agents, eg, calcium folinate.

To exert its antitumor activity, 5-FU must enter the target cells and interfere with one of two pathways: 1) metabolism to 5-fluorodeoxyuridine monophosphate (FdUMP), a potent inhibitor of the target enzyme thymidylate synthase (dTMPS)[3,4]; or 2) metabolism to 5-fluorouridine triphosphate (FUTP), which is subsequently incorporated into cellular ribonucleic acid (RNA) in place of the normal metabolite, yielding fraudulent RNA[5,6] (Figure 1). The relative importance of each pathway to the antitumor activity of 5-FU varies with different tumors.

Calcium Folinate Modulation of 5-FU

Calcium folinate is rapidly metabolized to various cofactors with 6R-LV, 5-LV, and 5-methyltetrahydrofolate (5-CH$_{3}$FH$_{4}$) being the dominant plasma cofactors. Significant differences in elimination plasma half-life exist among these metabolites. Because it has been demonstrated that both 5-LV and 5-CH$_{3}$FH$_{4}$ can modulate the cytotoxicity of 5-FU, it is not known whether each alone or both of these active metabolites play a major role in the intracellular accumulation of 5,10-methylenetetrahydrofolate (N$_{5}$,N$_{10}$CH$_{2}$FH$_{4}$).

The concept of modifying the therapeutic index of antimetabolites by administering noncytotoxic compounds dates back more than 30 years.[7,8] The conversion of deoxyuridine monophosphate (dUMP) into deoxythymidine monophosphate (dTMP) is carried out in two sequential chemical reactions: First, dTMPS catalyzes the substitution of the hydrogen in the C-5 position of dUMP with a methylene group; then, the enzyme reduces the methylene group to a methyl group. The cofactor that is involved both as the donor of the CH$_{3}$ group and, subsequently, as the reducing agent is the N$_{5}$,N$_{10}$CH$_{2}$FH$_{4}$.[9-11] Therefore, the conversion of uridylate into thymidylate involves the transient formation of a ternary complex constituted by the substrate dUMP, the enzyme dTMPS, and the cofactor N$_{5}$,N$_{10}$CH$_{2}$FH$_{4}$; with time and in the presence of insufficient concentration of folate cofactor, the ternary complex dissociates, releasing dTMP, the free enzyme, and dihydrofolate (FH$_{2}$), the oxidized cofactor. After administration of 5-FU, the FdUMP that is generated will compete with the physiologic substrate dUMP for dTMPS; however, due to the characteristics of the fluorine-carbon bond, the substitution of the fluorine atom with the methylene group will not occur and the reaction does not proceed further.

Activity of dTMPS, size of the cellular pools of dUMP, folate cofactor, and the amount of FdUMP generated are important determinants of response to 5-FU in the presence of calcium folinate. In particular, the presence of suitable amounts of the reduced folate cofactor in tumor tissues is critical, because a binary complex of FdUMP:dTMPS is relatively weak, whereas the ternary complex of FdUMP with dTMPS and N$_{5}$,N$_{10}$CH$_{2}$FH$_{4}$ is more stable and dissociates more slowly.

Key factors associated with the ability to potentiate 5-FU action by calcium folinate include
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stabilization of the ternary complex, which results in a pronounced and prolonged inhibition of the
target enzyme dTMPs and inhibition of DNA synthesis. The duration of dTMPs inhibition in the
presence of calcium folinate is also influenced by the extent of polyglutamation of N⁵,N¹⁰CH₂FH₄.

From the data generated to date, the rationale for the combination of 5-FU with calcium folinate can
be summarized as follows:

- Calcium folinate potentiates the cytotoxic effects of 5-FU primarily in tumor cells with
  relatively low intracellular concentrations of N⁵,N¹⁰CH₂FH₄, a folate cofactor essential for the
tight binding of FdUMP, the active metabolite of 5-FU, to the target enzyme dTMPs. Increased
cytotoxicity occurs due to stabilization of the ternary complex of
  N⁵,N¹⁰CH₂FH₄–dTMPs–FdUMP, which results in prolonged inhibition of dTMPs. Consequently,
  DNA synthesis inhibition is more rapid and sustained for a longer duration.

- The effect of calcium folinate was dose- and schedule-dependent. Cytotoxicity was optimal
  when 20 µmol/L of calcium folinate was exposed for 24 hours prior to 5-FU administration.
  Modulation of 5-FU does occur at lower concentrations, but, when these are used, the
  duration of calcium folinate exposure should be longer than 24 hours.

- In those tumor cells where incorporation of FUTP into cellular RNA was the primary site of
  5-FU action, calcium folinate shifted the drug effect to inhibition of deoxyribonucleic acid
  (DNA) synthesis via stabilization of the ternary complex.

- Polyglutamate forms of N⁵,N¹⁰CH₂FH₄ are more active potentiators of 5-FU cytotoxicity than
  monoglutamates. The extent of polyglutamate formation appears to be a function of the
  duration of exposure to calcium folinate.

Schedule of 5-FU Modulation by Calcium Folinate

Table 1 and Table 2 outline the various schedules of 5-FU/calcium folinate modulation under clinical
evaluation, including daily × 5, weekly × 6 (intravenous push and 24-hour infusion), and protracted
continuous intravenous infusion. The total 5-FU dose delivered per course of therapy was
approximately 1,275 mg/m² with the daily × 5 schedule; 3,000 mg/m² (intravenous push) or 15,600
mg/m² (24 hours) for the weekly schedule; and 9,000 mg/m² for protracted infusion. As discussed
below, therapeutic efficacy was achieved with a different profile of toxicity as a function of the
schedule but not the dose of calcium folinate.

Modulators of 5-FU Under Study

Table 3 is a list of the various 5-FU modulators, which include calcium folinate, modulator of FdUMP
to dTMPs binding (as discussed above); 5-ethynyluracil (776C85) (irreversible inhibitor of
dihydropyrimidine dehydrogenase [DPD], the enzyme responsible for degradation of 5-FU);
5-chlorodihydropyrimidine (5CDHP), a competitive inhibitor of DPD and a component of S-1, a new
5-FU prodrug; sodium oxonate, a competitive inhibitor of 5-phosphoribosyl-1-pyrophosphate
transferase, an enzyme responsible for the metabolism of 5-FU to fluorouridine monophosphate; and
interferon, a known modulator of 5-FU action. A summary of the site of action of these modulators is
presented in Table 4.

Clinical Trials

Phase III Trials in Colorectal Cancer

Randomized studies of 5-FU vs 5-FU/calcium folinate in patients with advanced colorectal cancer
indicate a response advantage for the combination over 5-FU alone in six of the seven trials, with
reported response rates of 20% to 30%.

Comparative Toxicity of 5-FU and 5-FU/Calcium Folate

Myelotoxicity

Where the treatments were designed to be equitoxic, the 5-FU/calcium
folinate arm was generally less myelotoxic than the 5-FU arm, or myelotoxicity was minor in
both arms. In all of the studies reported, thrombocytopenia was considerably less frequent
than leukopenia.

Diarrhea

Diarrhea was a more frequent side effect of 5-FU/calcium folinate than of 5-FU in
most studies; it was the major dose-limiting toxicity of patients treated with the Roswell Park
Cancer Institute weekly schedule and the weekly high-dose FU/calcium folinate reported by Köhne et al.[12]

- **Stomatitis**: Stomatitis was a prominent toxicity of the schedules in which 5-FU/calcium folinate was given on a daily × 5 schedule or in which the calcium folinate was given by continuous infusion. It was far less important as a toxicity in patients treated either with the Roswell Park or high-dose 5-FU/calcium folinate schedule.

**References:**


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