the phosphorothioates. In addition, chimeric oligomers, for example those that include phosphodiester and phosphorothioate or other linkages in their backbones, may prove to be active and stable and to have fewer nonsequence-specific properties than the all-phosphorothioates. And none of this discussion rules out the possibility that the all-phosphorothioate oligomers may have a very bright clinical future in their own right. Even though the antisense technology field is more than 10 years old, it is a curious and controversial area whose complexities we are just beginning to fathom.

References


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Biochemical Modulation of
Fluoropyrimidines: Is There an Optimal
(6R,S)Leucovorin Dose and Schedule?

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Over the past decade, a substantial base of clinical evidence has been developed supporting the effectiveness of combining the reduced folate cofactor (6R,S)leucovorin (LV) with fluorouracil (5-FU) in the treatment of colorectal cancer (1-7). Both in the advanced-disease setting and, more recently, in the postsurgical adjuvant treatment of primary colon cancer (8), benefit in terms of remission rate or disease-free survival has been demonstrated. However, despite the multiplicity of clinical trials, definitive data regarding the optimal dose, route, and schedule with which to deliver LV in combination with 5-FU remain elusive, since only a small number of randomized studies have attempted to examine this issue under conditions wherein the 5-FU dose and schedule have remained constant (3,9). Although the economic considerations that initially stimulated an examination of fluoropyrimidine biochemical modulation with "low-dose" LV have moderated, the disparity in toxicity profiles exhibited by the two commonly used treatment programs: dose-limiting diarrhea when 5-FU is combined with LV on a weekly bolus schedule (1) and mucositis with the daily times five bolus regimen (7).

Whereas the initial observation demonstrating an increase in fluoropyrimidine cytotoxicity toward murine leukemia cells in the presence of pharmacologic reduced folate levels was made 18 years ago (10), it is only during the past 5-6 years that preclinical investigations have begun to provide substantive insights into the biochemical mechanisms involved in the variable alterations in 5-FU cytotoxicity observed when LV is used on different schedules and at different concentrations both in vitro and in vivo (11-15). There is now little question that LV contributes to the therapeutic effect of the fluoropyrimidines after it is metabolized intracellularly to 5,10-methylenetetrahydrofolate polyglutamate(s) by enhancing the stability of the (enzymatically inactive) ternary complex formed by these polyglutamates with the 5-FU metabolite 5-fluorodeoxyuridine monophosphate (FdUMP) and thymidylate synthase (TS). In the absence of folate cofactors, FdUMP is only weakly bound to TS; the addition of folate polyglutamates, especially higher polyglutamated forms, dramatically increases the formation of the TS ternary complex, with a consequent increase in TS inhibition (16,17).

Recent studies (14,15) with the human colon cancer cell lines HCT-116 and NCI H630 have demonstrated that time of exposure, concentration, and interval between LV administration and treatment with 5-FU all play a critical role in modulating ternary complex formation, with polyglutamation of 5,10-methylene tetrahydrofolate and TS inhibition (after 5-FU addi-
tion) increasing for up to 18 hours after LV treatment. Using human colon xenografts, Houghton et al. (11,13) demonstrated that 5,10-methylenetetrahydrofolate polyglutamate levels in most tumors increased as a function of LV dose (range, 20-1000 mg/m²) and duration of exposure, with 24-hour infusions of LV (compared with bolus dosing or 4-hour infusions) producing the greatest increase in the level of higher polyglutamates. At equal doses of LV, longer infusions produced greater increases in 5,10-methylenetetrahydrofolate polyglutamates. Finally, Peters et al. (18) have recently found that, in humans, pretreatment with 500 versus 25 mg/m² of LV before a 500 mg/m² intravenous bolus dose of 5-FU produced a significantly increased degree of TS inhibition in surgical specimens of hepatic colon cancer metastases 48 hours after chemotherapy. It would appear, therefore, that the dose of LV is important for maximal TS inhibition. However, the available data also strongly suggest that the duration of reduced folate exposure plays a critical role in optimizing intracellular polyglutamate formation.

It is with these clinical and preclinical data in mind that the report by Cao et al. (19) in this issue of the Journal should be evaluated. The authors have examined both the toxicity and the efficacy of three clinically utilized fluoropyrimidine/LV schedules at both "high" and "low" doses of reduced folate using rats bearing the Ward colorectal carcinoma. When used at the maximally tolerated dose, the limiting toxicity of weekly 5-FU or 5-fluoro-2'-deoxyuridine (FdUrd) treatment was diarrhea; as expected from clinical observation, stomatitis was dose limiting, independent of the dose of LV, for the daily times four bolus and 96-hour infusion schedules. More important, however, are the observations that, for either 5-FU or FdUrd treatment, "high-dose" LV was the most effective on the weekly schedule and that the dose of LV was less important when the fluoropyrimidine was administered as a daily bolus or as an infusion. These results are consistent with the results of randomized clinical trials from both the Gastrointestinal Tumor Study Group (3) and the North Central Cancer Treatment Group (4,7) and suggest that when reduced folates are administered with fluoropyrimidines on an intermittent basis, higher folate doses are required to produce clinically significant alterations in intracellular folate polyglutamates. On the other hand, when LV is administered by continuous infusion (5,20,21) or by a daily bolus (25 mg/m²) that achieves a plasma concentration of greater than or equal to 1 μM (22), intracellular 5,10-methylene tetrahydrofolate polyglutamates accumulate, enhancing fluoropyrimidine cytotoxicity. Thus, the model system developed by Cao et al. (19) is clinically applicable, despite the presence of high levels of circulating thymidine in rats.

The current report also provides interesting information on the therapeutic activity of FdUrd in combination with LV. Although, as the authors point out, most FdUrd is rapidly converted to 5-FU when administered in a high dose, the antineoplastic activity of FdUrd with or without LV in this model system was significantly greater than that observed for 5-FU. The data suggest, and two phase I clinical trials support (23,24), the possibility that delivery of sufficiently high doses of FdUrd (a more proximate precursor of FDUMP than 5-FU) might lead to enhanced intracellular levels of FDUMP that, in the presence of LV, would enhance ternary complex formation. This is an interesting possibility that deserves careful phase II trial evaluation.

In summary, an appropriate, readily transplantable animal model now exists that appears to mimic the major features of each of the clinically useful fluoropyrimidine/LV dosing schemes. It is likely that the availability of this model system will allow significantly more rapid and predictive preclinical evaluation of novel, TS-directed biochemical modulation regimens in the future.

References


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**GETTING THE FACTS ON 5 A DAY**

How Americans are doing when it comes to fruits and vegetables

**Why eat five?**

As the link between diet and overall health continues to gain attention, public awareness of the benefits of fruits and vegetables has expanded. In a recent survey, 1,003 people were asked how likely they thought it is that eating fruits and vegetables can help reduce the risk of several health conditions. Perceived health benefits most frequently mentioned were:

- **Prevent Heart Disease**: 59%
- **Lose or Maintain Weight**: 64%
- **Prevent Cancer**: 48%
- **Lower Fat in Your Diet**: 75%

**Source:** National Cancer Institute

A National Cancer Institute Graphic