Enigma of Fluorouracil and Levamisole

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For the thousands of patients diagnosed each year with colon cancer, the NCI Consensus Conference statement in April 1990 recommending the use of fluorouracil (5-FU) and levamisole as surgical adjuvant chemotherapy in node-positive patients was a welcome advance in the treatment of this common malignancy (1). The announcement was based on the observation that the postoperative administration of 5-FU combined with levamisole significantly reduced the risk of dying within 3 years by 33% in patients with stage III colon cancer (2). This finding also led to an increased interest in studying the potential mechanisms of interaction between these two agents. Unfortunately, after 5 years of research, many questions are still unanswered concerning the basic pharmacology of this drug combination.

Careful in vitro studies of 5-FU and levamisole have failed to demonstrate a convincing cytotoxic interaction. At drug concentrations above 100 \( \mu M \), which are much higher than those achieved clinically, levamisole may have an additive cytotoxic effect against human colon cancer cells when combined with 5-FU (3). Kovach et al. (4) postulated that at high drug concentrations, levamisole or its metabolites may potentially interfere with cellular phosphatases, such as tyrosine phosphatase, ultimately leading to enhanced 5-FU cytotoxicity. However, at pharmacologically relevant drug concentrations up to 3 \( \mu M \) (5), levamisole by itself does not inhibit the growth of human colon cancer cells in vitro, and it has no demonstrable effects on 5-FU cytotoxicity (3). These findings have led to the proposal that the favorable effects of levamisole are due to the enhancement of the body's immune response rather than the biochemical modulation of 5-FU cytotoxicity. Further support for this theory comes from observations that 5-FU-based chemotherapy can induce a temporary state of immunosuppression in cancer patients (6). The role of levamisole as a stimulator of the immune system may also explain why this drug combination is no more active against advanced metastatic colon cancer than 5-FU alone (7). In situations where there is an overwhelming tumor burden, the potentiation of the suppressed immune system may be ineffective. Only in the presence of occult or microscopic disease, such as after surgical removal of the primary tumor, would the levamisole-induced stimulation of the body's antitumor immune system have any measurable impact on decreasing the risk of cancer recurrence. Unfortunately, consistent evidence for modulation of the immune response by levamisole at clinically relevant drug concentrations also has been difficult to demonstrate (8).

In this issue of the Journal, AbdAlla et al. (9) propose a potential mechanism of enhanced antitumor immunity that may explain the increased effectiveness of the combination of levamisole and 5-FU. In WiDr human colon cancer cells, the combination of 5-FU and levamisole enhanced human leukocyte antigen (HLA) class I protein expression due to a specific interaction at the level of messenger RNA (mRNA). In these cells, 5-FU by itself stabilized HLA class I mRNA turnover and increased the steady-state levels of this specific mRNA by 80%, presumably as a result of drug incorporation into RNA. At the same time, 5-FU also decreased the overall level of RNA synthesis. The addition of levamisole reversed the 5-FU-induced general inhibition of RNA synthesis and resulted in an additional increase of 70% in level of HLA class I mRNA compared with 5-FU alone. Because colon cancer cells may have decreased levels of HLA expression (9), this drug-induced enhancement in HLA class I expression may potentially lead to improved antitumor surveillance by the body's immune system, ultimately resulting in the decreased risk of cancer recurrence. This is an interesting and novel theory, but several important issues need to be considered further.

The key finding by AbdAlla et al. (9) was that levamisole reversed the generalized inhibition in RNA synthesis caused by 5-FU in this experimental system. This study reports the first biochemical or molecular interaction between these two agents to be demonstrated at pharmacologically relevant levamisole concentrations. Because 5-FU-induced alterations in RNA metabolism may be responsible for at least some of the drug's biologic effects (10), AbdAlla et al. postulated that levamisole may reduce the amount of clinical 5-FU toxicity caused by the inhibition of RNA synthesis (9). However, there is no evidence from clinical studies that the side effects of the 5-FU/levamisole regimen are any less than when 5-FU is given alone (11). In addition, even if the major interaction between these two drugs is to increase HLA class I expression, it is pure conjecture that this increase would lead to enhanced antitumor immunity and a decreased risk of tumor recurrence. Therefore, it is premature to suggest that adjuvant chemotherapy with a combination of 5-FU

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and levamisole could be improved by using lower doses of 5-FU that would stabilize HLA mRNA without inducing systemic toxic effects. Additional in vivo animal studies are necessary before these hypotheses are tested in a clinical trial.

Furthermore, in this in vitro study (9), cells were continuously exposed to 5-FU for 18-48 hours, followed by 6-12 hours of concurrent exposure to both 5-FU and levamisole. This pattern of exposure differs greatly from the most commonly used clinical schedule of drug administration in which levamisole is given for a 2-week treatment period and 5-FU for a 1-week treatment period. The impact of the combination schedule changes on the efficacy of this drug combination is unknown, and the effect of these potential alterations clearly needs to be better characterized in both preclinical and clinical studies. In addition, this article by AbdAlla et al. analyzed only a single cell line, and it is unproven whether the molecular interactions observed can be generalized to all colon tumors. As the authors state, levamisole can have varied effects on the expression of HLA antigens in different cancer cell lines, and it may actually decrease HLA expression in some tumors (12). Further validation of these results in additional colon cancer cell lines or, ideally, in actual tumor specimens would help to define the applicability of these findings to colon cancer in general.

The observations by AbdAlla et al. are also of interest because they raise important but unanswered questions concerning the mechanism of 5-FU- and levamisole-induced changes in RNA metabolism. In this study (9), the authors assumed that the effects of 5-FU on mRNA stability were the result of incorporation of the drug into mRNA. However, 5-fluorouracil metabolism on nucleic acids in general, and on mRNA in particular, are varied and complex and may not always be due to direct drug incorporation. For example, the regulation of translation of specific mRNAs, such as thymidylate synthase (TS) mRNA, is greatly affected by the presence of 5-FU metabolites, such as 5-fluoro-2'-deoxyuridine-monophosphate (FdUMP) and the normal nucleotide metabolite, deoxyuridine-monophosphate (dUMP), both of which accumulate in cells following 5-FU treatment. These metabolites directly interfere with the highly specific autoregulatory binding of TS protein to its own mRNA and result in an increase in efficiency of TS mRNA translation (13). Interactions of 5-FU metabolites with TS may also affect the synthesis of other cellular proteins such as c-myc (14). Thus, 5-FU can alter important RNA-protein interactions independent of its direct incorporation into nucleic acid. Furthermore, even very high levels of 5-FU incorporation into mRNA may not always result in a nonfunctioning molecule. The complete substitution of 5-FU for uracil in mature TS mRNA did not alter the efficiency or fidelity of its translation in vitro protein synthesizing systems (15,16). Alternatively, 5-FU may interfere with the processing of precursor mRNA by incorporating into small nuclear RNA (snRNA), such as the U2, U4, or U6 snRNA, which are critically involved in RNA splicing reactions (17,18). However, AbdAlla et al. are correct in their suggestion that drug-induced alterations in RNA metabolism may represent an important and commonly overlooked mechanism for antimetabolite action.

Ultimately, these issues may be overshadowed by the greater question of whether actual true clinical synergy exists for 5-FU and levamisole. The contribution of levamisole to the adjuvant treatment of colon cancer has been questioned by other investigators (19); currently, no well-designed trial has compared the combination regimen with a 5-FU-alone control arm. In the only randomized study directly comparing the combination of 5-FU and levamisole with 5-FU alone as adjuvant therapy for resected colon cancer (20), a significant survival advantage was found for the combination regimen; however, this study included only a small number of patients with stage III disease (41 patients randomly assigned to three treatment arms) and it utilized a relatively unreliable oral method of 5-FU administration. This debate has been argued extensively elsewhere (1,5,8,11,19); it will not be recapitulated here, but it does emphasize our poor understanding of this drug combination.

Nonetheless, the use of 5-FU and levamisole in the surgical adjuvant treatment of node-positive colon cancer is an important advance because it validates the use of adjuvant chemotherapy in this disease. However, the enigma concerning the exact nature of the pharmacologic interaction of 5-FU and levamisole remains unresolved. The article by AbdAlla et al. (9) expands our understanding of how these agents may potentially interact at the molecular level, but fundamental questions still remain. Perhaps the final and best resolution for this perplexing dilemma will be for it to become less relevant. As newer strategies for the adjuvant chemotherapy of colon cancer are tested, even more effective treatments may emerge. Currently, regimens with proven activity in metastatic colon cancer, such as the combination of 5-FU with the biochemical modulator, folinic acid, are being examined in clinical trials containing a 5-FU/levamisole control arm (21). As the results of these ongoing studies become available, the question as to the exact nature of the interaction of 5-FU and levamisole may become less pertinent, and, ultimately, less vexing to cancer chemotherapy pharmacologists.

References

Selenium and Cancer: Risk or Protection?

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The cohort study by Garland et al. (1) in this issue of the Journal tests the selenium (Se) and cancer hypothesis using adjusted toenail Se concentration as a surrogate measure for tissue Se. It is an apparent tour de force of cohort analysis, based on a cohort of 62,641 nurses who provided toenail clippings in 1982 and were then followed prospectively for 41 months. The 503 nurses who developed cancer other than breast cancer or nonmelanoma skin cancer between 1982 and 1986 were matched to nurses who had not developed cancer. No significant associations were observed in the unadjusted analyses; however, trends toward a higher incidence of cancer were suggested for increasing toenail Se levels in the adjusted analyses that included and excluded breast cancer cases.

In 1969, Shamberger and Frost (2) first proposed that the geographic distribution of the essential nutrient Se in the United States was inversely associated with cancer mortality rates. Since this initial report, numerous experimental and epidemiologic studies have investigated the hypothesis that enhanced Se status reduces the risk of cancer. el-Bayoumy (3) has reviewed the experimental studies.

Several biologic mechanisms have been proposed to explain how supplementation with Se could reduce the incidence of a number of different cancers. The proposed mechanisms (4,5) involve the antioxidant potential of the Se-dependent glutathione peroxidase enzyme system, effects on cellular immune response, carcinogen metabolism, carcinogen-DNA binding, and apoptosis.

Geographic and ecologic studies of cancer mortality patterns can establish priorities for evaluating the cancer prevention potential of Se in humans for each specific type and site of cancer. Our study (6) of cancer mortality and forage Se levels in U.S. counties have shown a relatively consistent pattern of excess cancer mortality within the regions of the United States that have lower Se compared with those regions that have variable or adequate Se levels. This association is similar for both males and females and for each of the major cancer sites examined, except prostate cancer.

The majority of prospective epidemiologic studies rely on measurement of Se in serum and plasma, rather than in toenails, to reflect tissue Se concentrations. The results and statistical significance of these studies vary, in part because of the small number of cases in each cohort. Comstock et al. (7) have compared several of these prospective cohort studies to evaluate both the magnitude and consistency of the inverse association between cancer and serum retinol, beta carotene, alpha tocopherol, and Se. The results of this analysis suggest that of the nutrients studied, Se may have the most consistent inverse association with cancer incidence in these cohorts.

The Linxian cancer prevention trials reported in this Journal (8, 9) included individuals living in a region of China with extremely high rates of esophageal and stomach cancer. The results showed that the treatment arm containing Se, beta carotene, and alpha tocopherol had significantly fewer cases of, and mortalities from, stomach cancer compared with the placebo and the other treatment arms. Currently, Se is the most promising of these three nutrients, in part due to the recently published (10) null results for the primary end point of lung cancer in the Finnish cancer prevention trial using beta carotene and alpha tocopherol. Further evaluation of these agents in lower-risk populations with longer periods of treatment and follow-up may...