LETTERS

Antitumor Drug Interactions: Additional Data

In response to the article "Cancer chemotherapy: An unexpected drug interaction" by Thomas H. Maugh II (Research News, 15 Oct., p. 310), we would like to call attention to published data (*1*) from our laboratory presented at the annual meeting of the American Association for Cancer Research in Toronto in March 1976. The data clearly show an unfavorable interaction between methotrexate and 5-fluorouracil with respect to their antimetabolic effect in de novo DNA synthesis. These studies were done in the L1210 cell system and more recently confirmed in the Friend leukemia system and in human bone marrow. We utilized the deoxyuridine suppression assay and tritiated deoxyuridine incorporation into DNA as sensitive indicators of de novo DNA synthesis which can measure the effects of 7.5×10^{-9} molar methotrexate and 0.2 microgram of 5-fluorouracil per milliliter (*2*). The combination of 5-fluorouracil and methotrexate added to the cell lines or bone marrow did not significantly increase the de novo DNA defect as compared to the same amount of 5-fluorouracil or methotrexate alone (Table 1). The anti-DNA effect of methotrexate in this system could be significantly reduced by washing the cell lines after drug exposure, whereas the effect produced by 5-fluorouracil was not significantly reduced by washout. However, washing of the cells following exposure to the combination of the two drugs resulted in a 70 percent loss of the 5-fluorouracil effect. This decrease of 5-fluorouracil effect was directly related to the concentration of methotrexate and occurred even in sequential exposure. The addition of 5-formyltetrahydrofolic acid (folinic acid) to the drug combination appeared to prevent the washout of the 5-fluorouracil effect. We interpreted these results to mean that (i) some methotrexate–5-fluorouracil combinations are not additive or synergistic; (ii) methotrexate may diminish 5-fluorouracil effect during washout by preventing expression of 5-fluorouracil action mediated through a 5-fluorouracil high-affinity site; and (iii) the loss of 5-fluorouracil effect in the presence of methotrexate probably resulted from a deficiency of the necessary folate coenzyme for the methylation of deoxyuridylate.

Our findings are supported by those of Santi and Martin, who have shown that methotrexate can interfere with the activ-

Table 1. Methotrexate (MTX) and 5-fluorouracil (5-FU) interaction in L1210 leukemia. Cells were first incubated for 3 hours at 37°C in Hanks' balanced salt solution. Some were then washed three times with cold Hanks' solution. After the washout, [^{3}H]deoxyuridine (0.1 μc per tube) was added, and the incubation continued for 90 minutes. Additional details are given in (*2*).

Additions	[^{3}H]Deoxyuridine incorporated into DNA	
	No washout (%)	Washout (%)
Saline	100.0	100.0
MTX, 10^{-6} *M*	17.4	95.6
MTX, 10^{-7} *M*	90.0	105.1
5-FU, 3.1 μg/ml	6.9	13.9
5-FU, 0.78 μg/ml	37.1	22.2
MTX, 10^{-6} *M* + 5-FU, 0.78 μg/ml	15.8	60.8

ity of 5-fluorodeoxyuridine when given in combination. Similarly, we and others have demonstrated that the cellular uptake of methotrexate and its antimetabolic effect may be reduced by 50 percent in the presence of certain corticosteroids commonly used in combination chemotherapy (*2*, *3*). These studies emphasize the need for careful pharmacologic and biochemical assessments of the interaction of cancer chemotherapeutic agents when used in combination.

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We also have been concerned about the use of methotrexate and 5-fluorouracil in combination in the clinic and about the conflicting evidence as to whether this combination of drugs is synergistic, additive, or antagonistic (*1*). We hypothesized that the combination would give additive or synergistic antitumor effects if treatment with high doses of methotrexate preceded treatment with 5-fluorouracil, and that this combination would be antagonistic if the drugs were administered in the reverse sequence. The rationale for this theory was that pretreatment with methotrexate would result in high intracellular levels of this drug which would act as an analog of

N^5,N^{10}-methylenetetrahydrofolate, the coenzyme for thymidylate biosynthesis, and as a result 5-fluorodeoxyuridine monophosphate would bind irreversibly in ternary complex with methotrexate to thymidylate synthetase (*2*). The reverse sequence would not result in irreversible inhibition, since 5-fluorodeoxyridine monophosphate levels would be falling at a time when methotrexate levels would be increasing. Use of high doses of methotrexate would also be important, since low intracellular levels of methotrexate would inhibit dihydrofolate reductase, but not thymidylate synthetase, thus leading to a decrease in N^5,N^{10}-methylenetetrahydrofolate levels, and possible antagonism with 5-fluorouracil.

We have shown (*3*) that, in accord with this concept, treatment of mice bearing the sarcoma 180 with methotrexate 1 to 4 hours before treatment with fluorouracil enhances antitumor effects of fluorouracil; when the combination is given simultaneously, no additive effects are present; when treatment with 5-fluorouracil precedes treatment with methotrexate the effect is antagonism (less effect than either drug alone). Martin *et al.* (*4*) have also reported that methotrexate pretreatment (1 hour) followed by treatment with 5-fluorouracil resulted in therapeutic synergy against a spontaneous (CD8FI) mammary cancer.

In light of these results we have instituted clinical studies using pretreatment with methotrexate in combination with treatment with 5-fluorouracil in patients with breast cancer and colon cancer. If the experimental tumor data are applicable to human cancer, this should lead to an increased therapeutic effect without an increase in drug toxicity.

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Maugh refers to an observation of Santi and Martin that methotrexate interferes with the activity of 5-fluorodeoxyuridine on tumor cells grown in culture when the drugs are given at the same time. He also indicates that methotrexate should interfere with the activity of 5-fluorouracil, since the mechanism of action is similar to that of 5-fluorodeoxyuridine. In his article, Maugh states that Martin and Santi contacted the National Cancer Institute, and that from limited data it was concluded that no additive effects were observed when methotrexate and 5-fluorodeoxyuridine were employed in combination chemotherapy of tumor-bearing mice.

In view of this, we should like to call attention to studies involving combination chemotherapy with methotrexate plus 5-fluorouracil in the treatment of mice with advanced leukemia L1210 (*1*). In a series of five experiments, Kline *et al.* observed that concomitant daily treatment with methotrexate plus 5-fluorouracil was more effective in increasing the survival time of the mice than daily treatment with the drugs employed individually. In these experiments the drugs were combined in a number of methotrexate/5-fluorouracil dosage ratios, and a wide range of daily treatment levels was employed for each combination ratio. Daily methotrexate alone was consistently more effective than daily 5-fluorouracil alone in increasing survival time, and the optimal combination treatment was 11 to 57 percent more effective than the optimal daily dose of methotrexate alone. In these experiments the improved therapeutic effect was observed with a relatively low daily dose of 5-fluorouracil combined with a dose of methotrexate which was optimal or higher than the optimal daily dose for methotrexate alone. The data suggest that, on the daily schedule, 5-fluorouracil contributed to the enhanced therapeutic effect without itself adding significantly to the toxicity for the host. Other schedules of therapy for which a therapeutic advantage was obtained with this drug combination include 5-fluorouracil administered as a single dose (day 6 after leukemic inoculation) plus methotrexate administered either every 4 days or daily (from day 6).

In another experiment (*2*), a comparison was made of concomitant daily treatment with methotrexate plus 5-fluorouracil and methotrexate plus 5-fluorodeoxyuridine in the treatment of mice with advanced leukemia L1210. Both drug combinations were approximately equally effective in enhancing therapy relative to the drugs employed individually.

The selection for combination chemotherapy of methotrexate plus 5-fluorouracil (and 5-fluorodeoxyuridine) was based on the biochemical rationale that therapeutic enhancement might result

from a sequential blockade in thymidylate synthesis or as a form of concurrent blockade involving purine and thymidylate biosynthesis. However, although therapeutic enhancement was obtained with the combination, it was concluded that the fundamental nature of the enhanced therapeutic response remained to be determined. Thus, although in accordance with the rationale, an "expected drug interaction" occurred, the fundamental basis for the therapeutic enhancement may not have been related at all to this interaction.

A wide variety of combinations of drugs has been demonstrated, in animal tumor systems, to provide a therapeutic advantage over that observed with the drugs employed individually. The demonstration of such therapeutic synergism may be dependent upon the dosage ratios employed, the dosage levels, and the schedule of administration, including the interval between treatments, number of treatments, total duration of treatment, and the timing of administration of the drugs relative to one another. The degree of advancement of disease and extent of infiltration or metastasis, as well as a variety of factors pertaining to the host, may influence the extent of therapeutic effect observed. Any biochemical rationale pertaining to fundamental interactions of a drug combination must be reflected in increased antitumor specificity in the tumorous host in order to obtain an improved therapeutic response.

We agree with Maugh that detailed investigations of drug combinations should be conducted and that fundamental investigations of biochemical and pharmacologic action, both in vitro and in vivo should be pursued, both retrospectively and prospectively, in relation to the usefulness of drug combinations in the treatment of clinical neoplasia.

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Mailing Labels: Hot Air, Patience, and Other Suggestions

Eugene E. Hinman (Letters, 12 Nov., p. 674) may remove the labels from *Science* covers by the application of heat from a hot air blower (a small hair dryer works very nicely) for about 15 seconds and then carefully peeling off the label. A hot iron will probably work equally as well, but water will not work, since the glue used on the label is not water-soluble. The heat treatment process produces a *Science* cover in mint condition, provided the Postal Service has not already mutilated it.

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JOHN GRAHAM
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