Effect of Tetrahydrohomofolate on DNA Biosynthesis in Leukemic Mice[1] (35973)

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Early studies of Mead *et al.* (1) demonstrated that tetrahydrohomofolic acid was relatively more effective against the methotrexate-resistant tumor than the parent sensitive leukemia L1210. Based on the findings of Goodman *et al.* (2) that dihydrohomofolic acid was as effective a substrate of dihydrofolate reductase as dihydrofolic acid, and the product of reduction was a good inhibitor of *Escherichia coli* thymidylate synthetase, it was considered likely that the drug might inhibit DNA biosynthesis which could account for its antitumor activity. However, Livingston *et al.* (3) were not able to show inhibition of DNA synthesis from tritiated deoxyuridine in leukemic spleen suspension following incubation with tetrahydrohomofolic acid. It was important, therefore, to establish whether this lack of effect *in vitro* was observed in intact animals since host-mediated effects are sometimes not demonstrable *in vitro*. In the present study, an attempt has been made to determine whether the effect of tetrahydrohomofolic acid on the uptake of ^{14}C-orotic acid and ^{14}C-formate into DNA 16 hr after the drug administration, can be correlated with the antileukemic effectiveness of the drug in mice bearing advanced methotrexate-sensitive L1210 and -resistant L1210/FR-8.

Materials and Methods. ^{14}C-6-Orotic acid (60.8 mCi/mmole) and ^{14}C-formate (25 mCi/mmole) were obtained from Amersham/Searle Corporation, Des Plaines, Illinois. Tetrahydrohomofolic acid (H_4HF, NSC-89,473) was obtained from Cancer Chemotherapy National Service Center, National Cancer Institute.

Leukemias L1210 and L1210/FR-8 were inoculated subcutaneously (sc) into male BDF_1 mice (10 to 12 weeks old) according to the procedure described by Goldin *et al.* (4). The L1210/FR-8 (dichloromethotrexate and methotrexate-resistant) is characterized by high levels of dihydrofolate reductase (5). H_4HF was dissolved in 2% sodium bicarbonate containing 0.6% ascorbate for administration. To study the effect of a single dose, mice bearing an 8-day-old sc tumor were selected. H_4HF (400 mg/kg) was given intraperitoneally (ip) (4 mice/group) 30 min before the administration of radioactive precursors (250 μCi/kg, ip) and the mice were sacrificed after 16 hr. Liver, kidney, and spleen were pooled, homogenized (25%; w/v) in cold water and three aliquots were analyzed for DNA according to the procedure of Wannemacher *et al.* (6). To determine radioactivity, 0.4 ml of the DNA extract was added to 10 ml of Bray's scintillator solution (7) in plastic vials and counted in a Packard scintillation spectrometer. The efficiency of counting was 75%. To study the effect of daily doses, mice bearing a 5-day-old tumor were injected with 400 mg of H_4HF/kg daily for 4 days. The radioactivity was injected 30 min after the last dose; and the mice were sacrificed after 16 hr.

Results and Discussion. Table I shows that a single dose of H_4HF did not cause demonstrable inhibition of orotate and formate incorporation into DNA in organs of leukemic mice. However, when the drug was given daily for 4 days, marked inhibition of uptake of

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TABLE I. Effect of Tetrahydrohomofolic Acid (H_4HF) on Uptake of Labeled Precursors into DNA of Organs of Leukemic Mice.[a]

Tissues	Control (cpm/mg × 10^{-3})	H_4HF 400 mg/kg × 1 (cpm/mg × 10^{-3})	H_4HF 400 mg/kg × 1 % of control	H_4HF 400 mg/kg × 4 (cpm/mg × 10^{-3})	H_4HF 400 mg/kg × 4 % of control
^{14}C-Formate					
L1210					
Spleen	8.6	8.8	102	8.2	95
Liver	9.1	9.6	105	9.4	103
Kidney	7.6	9.5	125	6.8	89
L1210/FR-8					
Spleen	17.0	11.9	70	5.4	31
Liver	15.7	10.1	67	7.4	47
Kidney	10.3	9.9	96	5.0	49
^{14}C-Orotic Acid					
L1210					
Spleen	2.6	3.6	138	2.8	107
Liver	2.8	3.0	107	4.0	142
Kidney	1.1	1.6	145	1.2	109
L1210/FR-8					
Spleen[b] 2 hr	2.9	2.1	72		
4 hr	3.9	4.5	115		
8 hr	3.6	4.9	136		
16 hr	1.5	1.4	93	0.8	53
Liver	1.2	1.3	108	0.4	33
Kidney	3.3	4.0	121	0.8	24
Nonleukemic mice	1.6			1.3	81

[a] Radioactive precursors were injected 30 min after the last dose of H_4HF and the animals were sacrificed after 16 hr. Average value of 3 aliquots is presented.

[b] Animals were sacrificed at 2, 4, 8, and 16 hr after drug injection.

both the precursors into DNA was noted in L1210/FR-8 tumor-bearing mice but not in L1210 leukemic or nonleukemic mice. In spleen, liver, and kidney, the formate incorporation into DNA was, respectively, 31, 47, and 49% of the control values. For orotate, the respective values were 53, 33, and 24%. Since L1210/FR-8 is more responsive to H_4HF therapy than L1210 (1), an apparent correlation between the antitumor activity of the drug and inhibition of DNA biosynthesis can be seen. The relationship was not apparent after the administration of a single dose because it is likely that the effect of a single dose was slight and of short duration. In fact, inhibition of orotate incorporation into DNA from spleen of mice bearing L1210/FR-8 at 2, 4, and 8 hr after a single dose of H_4HF was found to be less than 30%.

Liver, spleen, and subcutaneous tumor of L1210/FR-8 leukemic mice contain 20- to 30-fold higher levels of dihydrofolate reductase compared to the respective tissues of L1210 leukemic mice (5). Mishra *et al.* (8) have shown that dihydrofolate reductase is required to maintain H_4HF in its fully reduced form in liver. When methotrexate was given to block the enzyme, substantial amounts of dihydrohomofolic acid (H_2HF) accumulated in the organ. Further studies (9) have shown that H_2HF is reduced to H_4HF in mice, and that the antitumor activities of both these compounds against the L1210/FR-8 tumor were comparable. H_2HF, like H_4HF, was also found to be less effective against leukemia L1210 than L1210/

FR-8. If it is assumed that H_4HF is the active form of the drug it could follow that the dihydrofolate reductase level in tissues or leukemic cells may be a determining factor in the biological activity of the drug. The differential effects of H_4HF on DNA biosynthesis observed in this study support the above premise.

Summary. A relationship has been noted between the antitumor activity of H_4HF and its effect on incorporation of formate and orotate into DNA of organs of mice bearing advanced methotrexate-sensitive (L1210) and -resistant (L1210/FR-8) leukemia after daily treatment with H_4HF for 4 days. Both formate and orotate uptake into DNA was markedly inhibited in organs of mice bearing L1210/FR-8 leukemia, but not in mice bearing L1210 leukemia. Single doses did not cause demonstrable inhibition of DNA synthesis in organs of mice bearing either of the tumor lines.

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