Effect of Folate-deficient Diet and 5-Fluorouracil Treatment in the *in vivo* Model of Mice L1210 Leukemia

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Summary

The present study investigated the effect of reduced folic acid content in the organism, due to deficient diet, on the survival time of mice with L1210 leukemia, treated with fluorouracil. One of the mechanisms of the cytotoxic effect of fluorouracil is the inhibition of thymidylate synthase by binding fluorodeoxyuridylate, a 5-fluorouracil metabolite. Tetrahydrofolate factor, for which folic acid is the substrate, takes part in this process. From the presented study it follows that the synthetic diet prepared without the addition of folic acid caused significantly lower blood serum folic acid levels, reduction of vitamin B₁₂ level, anemia, as well as lowered leukocyte count. The antineoplastic effect of fluorouracil on L1210 leukemia was in that group of mice weaker than the effect of fluorouracil in mice receiving synthetic diet which met their folic acid requirement.

Key words : 5-fluorouracil, Mice L1210 leukemia, Experiment *in vivo*, Folic acid deficiency, Synthetic diets

Introduction

5-Fluorouracil is among the most effective drugs in the treatment of adenocarcinomas of the gastrointestinal tract, breast cancer and squamous cell carcinoma of the head and neck (1). FU may also be converted by appropriate modulation to an active drug in neoplasms in which they have little or no single agent activity : leukemias, lymphomas. *In vitro* studies have shown marked potentiation of FU cytotoxicity by methotrexate as well as by folates against a human lymphoblastic leukemia cell line CCRF-CEM (2). Antineoplastic activity of FU is complex; one of the mechanisms of the cytotoxic effect of the drug is the inhibition of thymidylate synthase by binding FdUMP, FU metabolite. Tetrahydrofolate cofactor, 5, 10-methylenetetrahydrofolate, for which fol-

ic acid is substrate, takes part in this process (3).

The presented study investigated the effect of reduced folic acid content in the organism, due to deficient diet, on the survival time of mice with L1210 leukemia treated with FU.

Materials and Methods

Animals and diets

The experiments were carried out on male mice of BDF₁ strain, weighing 18~20 g (10 weeks old at the beginning of the experiments), obtained from the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences (Wrocław), quarantined for 7 days after arrival. Mice divided into 2 groups, fed during the whole period of the experiments with two different laboratory diets. Group 1 received a synthetic diet A requirements for protein, fats, carbohydrates, microelements and vitamins, prepared according to Urbanek-Karlowska (4, 5). Diet A contained 2 mg of folic acid per kilogram of diet. Group

Abbreviations

FU-fluorouracil, FdUMP-fluorodeoxyuridine monophosphate, FUTP-fluorouridine triphosphate, dTMP-thymidine monophosphate, MST-median survival time, ILS%-increase life span over control untreated.

2 was fed with a synthetic diet B, prepared without folic acid.

Antineoplastic effects on L1210 leukemia in mice

The assessment of antineoplastic activity of FU, was carried out according to the method of Geran *et al.* (6). L1210 leukemic cells, passaged in DBA/2 mice, were transferred i.p. into BDF₁ experimental animals, divided into 2 groups (3×10^5 L1210 cells per mouse). Prior to the inoculation with leukemic cells, the BDF₁ mice had been fed with 2 different synthetic diets for 14 days. The day of inoculation was regarded as the "zero" day of the antileukemic assay. FU was injected i.p. 24 h after inoculation with L1210 leukemia, as single 80, 40, 20 mg/kg doses in two experimental groups fed with different diets. Control mice inoculated with L1210 leukemia in both groups did not receive any drugs. FU (Fluoroblastin) from Farmitalia was used for the assay.

The prolongation of survival time of mice subjected to FU treatment, as compared with control ones, was calculated according to the following formula :

$$ILS\% = \left[\frac{MST_t}{MST_c} - 1 \right] \times 100$$

where ILS%—prolongation of survival time of treated mice

MST_t—median survival time of mice in the treated experimental group

MST_c—median survival time of mice in the control, untreated group

ILS% was used as the criterium of antineoplastic effect. Prolongation of the survival time of treated animals, in comparison with the untreated group, by at least 25%, is an indicator of antineoplastic effect of the used dose of the drug. Median survival time of the animals was calculated according to the following formula :

$$MST = \frac{x+y}{2}$$

where x—the earliest day when the number of dead animals amounts to $\frac{N}{2}$

y—the earliest day when the number of dead animals amounts to $\frac{N}{2} + 1$

N—number of animals in the group

Hematological parameters assay

Erythrocyte and leukocyte counts, as well as he-

Table 1. Effects of FU on the survival of mice with leukemia L1210 after 14 days of folic acid-replete and folic acid-deficient diets.

Folic acid-replete diet A

Therapeutics

FU (mg/kg)	MST (days)	$\bar{X} \pm SE^*$ (days)	ILS %
0	8.0	8.5 ± 0.2	0.0
20	9.5	$9.7 \pm 0.3^*$	18.7
40	10.0	$10.0 \pm 0.4^*$	25.0
80	13.0	$13.2 \pm 0.3^*$	62.5

Folic acid-deficient diet B

Therapeutics

FU (mg/kg)	MST (days)	$\bar{X} \pm SE^*$ (days)	ILS %
0	8.0	8.5 ± 0.2	0.0
20	8.0	$8.0 \pm 0.3^*$	0.0
40	8.5	$8.5 \pm 0.2^*$	0.0
80	9.0	$9.0 \pm 0.4^*$	12.5

matocrit and hemoglobin values were determined in the experimental BDF₁ mice by means of standard laboratory methods. Blood samples were obtained from the cardiac ventricles of animals in light ether aethylicus anesthesia and collected into heparinized capillary tubes. Erythrocyte counts were done using a Thome hemocytometer, and leukocyte counts using a Burkner hemocytometer. Hemoglobin concentrations were determined by means of the standard cyanmet-hemoglobin method. Blood samples were collected prior to initiation of the diets, as well as after 14 days of feeding with the synthetic diets. Determination of concentrations of folic acid and vitamin B₁₂ was done by means of the radioisotopic method using : Radioassay Vitamin B₁₂/Folate (Farmos Diagnostica-Finland).

Statistical analysis

In all the cases the normality of distribution was checked by means of Kolmogorow-Smirnow test with correction by Lilliefors and equality of variance by means of Fischer's test. As the obtained variances were identical, Student's t-test was used. The presumed level of significance was 0.05.

Results

Table 1 presents the antineoplastic effects of FU in mice with L1210 leukemia after feeding with syn-

Table 2. Folate, vitamin B₁₂ levels in plasma and hematological parameters in mice after 14 and 28 days of folic acid-replete and folic acid-deficient diets

Parameters	Folic acid – replete diet A		Folic acid – deficient diet B	
	14 days	28 days	14 days	28 days
Folate(μg/L)	10.79 ± 2.11	12.13 ± 1.37	4.09 ± 0.77*	2.5 ± 0.48*
Vit B ₁₂ (ng/L)	1139.76 ± 135.71	1014.90 ± 172.80	696.38 ± 46.63	521.6 ± 102.5*
Hematocrit (%)	54.90 ± 1.30	54.00 ± 0.79	49.10 ± 1.30*	40.25 ± 1.38*
Hemoglobin (g/dL)	14.30 ± 0.10	13.25 ± 0.40	13.70 ± 0.10*	12.50 ± 0.76*
Erythrocytes (×10 ¹² /L)	6.92 ± 0.27	6.80 ± 0.99	6.19 ± 0.17*	5.07 ± 0.17*
Leukocytes (10 ⁹ /L)	6.50 ± 0.51	5.92 ± 0.35	4.88 ± 0.46*	4.45 ± 0.48*
Platelets (×10 ⁹ /L)	3.03 ± 0.35	3.08 ± 0.16	2.90 ± 0.40	2.98 ± 0.12

Experimental conditions were as described in Materials and Methods. Six animals in each group. Values are means ± SE.

*Significantly different at $p < 0.05$ between group diet A and diet B fed.

thetic diets containing various levels of folic acid.

FU administered to mice inoculated with L1210 leukemic cells after 14-day period of feeding synthetic-replete diet with folic acid content 2 mg/kg diet caused a prolongation of survival time in comparison with that of control, untreated animals. The increase of survival time was dependent on the administered dose. FU therapy administered to mice inoculated with L1210 leukemia after 14-day period of feeding with synthetic diet without folic acid content using analogous drug doses as those used in the previous group, did not cause significant increase of survival time in any group.

No differences in survival time were found, however, among the groups of untreated mice inoculated with L1210 leukemia fed with various synthetic diets. Table 2 presents the effects of 14-day and 28-day periods of feeding with synthetic diets containing various folic acid levels on selected hematological parameters, as well as on blood serum levels of folic acid and vitamin B₁₂ in mice.

Feeding with synthetic diet 8 without folic acid content led to a decrease of hemoglobin, hematocrits, erythrocyte and leukocyte count values. Blood serum folic acid and vitamin B₁₂ levels were reduced in comparison with the values observed in the blood serum of mice receiving the diet A which met the animals requirement for folic acid.

Discussion

From the presented study it follows that the synthetic diet prepared without the addition of folic acid

caused significantly lower blood serum folic acid levels, reduction of vitamin B₁₂ level, anemia, as well as lowered leukocyte count. The antineoplastic effect of FU on L1210 leukemia was in that group of mice weaker than the effect of FU in mice receiving synthetic diet which met their folic acid requirement. FU exerts its cytotoxic effect mainly by two mechanisms inhibition of RNA synthesis by binding of FUTP (FU metabolite), as well as inhibition of DNA synthesis by depletion of dTMP pool, which results from the inhibition of thymidylate synthase another metabolite of FU, FdUMP. Binding of FdUMP to the enzyme requires the activity involvement of tetrahydrofolate cofactor, for which folic acid is the substrate (3, 7, 8). Depletion of the tetrahydrofolate cofactors pool may be expected as results of decreased availability of folic acid. FU monotherapy has been routinely used in the treatment of patients with advanced stages of carcinoma of the colon and rectum with diagnosed metastases. The low efficacy of the therapy, limited to only 15~20%, is due mainly to the advanced stages of the disease (9). Deficient nutrition of such patients may lead to the deficit of folic acid in the organism and consequently to lower cytotoxic potency of FU (8). In malignancies, folic acid requirement is increased (10). Until quite lately it was believed that folate deficiency might be beneficial in patients and animals with neoplasms, while administration of larger quantities of folic acid might have cocarcinogenic effect (11). But now, however, a considerable evidence has been accumulated, which indicates that normal cells showing deficiency of this vitamin are more susceptible to neoplastic transfor-

mations (11, 12, 13). Thus determination of folic acid levels in the neoplastic patients prior to administration of FU seems plausible. Folic acid supplement might increase the therapeutic efficacy of FU.

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