KINETICS OF PROLIFERATION OF MOUSE MAMMARY GLAND ADENOCARCINOMA CELLS UNDER THE INFLUENCE OF METHYLCOBALAMINE

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The mechanism of the stimulating action of methylcobalamine on growth of adenocarcinoma 755 in mice was investigated. The significantly more rapid growth of adenocarcinoma 755 under the influence of the cobalamine coenzyme was shown to be determined by an increase in the proliferative pool, while parameters of the mitotic cycle and minimal mortality of the tumor cells remained stable. Potentiation of the antitumor action of methotrexate, when given in combination with methylcobalamine, is evidently due to inhibition of DNA synthesis in a much greater subpopulation of cells in the S-phase.

KEY WORDS: proliferation of mouse mammary gland adenocarcinoma cells; methylcobalamine; methotrexate.

To increase the effectiveness of combined chemotherapy of certain transplantable animal tumors the writers have used a new class of compounds – cobalamine derivatives. Following combined administration of methylcobalamine and its analogs with methotrexate a significant increase was found in the inhibition of growth of transplantable tumors: mammary gland adenocarcinoma 755, RShM-5 carcinoma of the cervix uteri, and also prolongation of the life span of mice with L 1210 leukemia [1, 3].

In this connection it was decided to study the role of the cobalamine coenzyme and its analogs in potentiating the antitumor activity of methotrexate. This paper gives the results of investigations of the effect of methylcobalamine on the kinetics of growth and proliferation of mouse mammary gland adenocarcinoma cells.

EXPERIMENTAL METHOD

A mammary gland adenocarcinoma was transplanted into female C57BL mice (60 animals weighing 20-25 g) by subcutaneous injection of a suspension of tumor cells in 0.5 ml medium No. 199. On the 2nd and 6th days after transplantation of the tumor, the mice of the experimental group were given methylcobalamine in a dose of 10 μg/kg by intramuscular injection.

The kinetics of proliferation of adenocarcinoma 755 cells was studied by an autoradiographic method using thymidine-^{3}H on the 8th day of growth of the tumor, 24 h after the end of methylcobalamine administration. Attention was paid to the duration of the mitotic cycle (Tc) and of its individual periods ($t_{g_1}^{+}t_M$; t_S; t_{g_2}), the proliferative pool (Pc), and the cell loss factor (φ) in the tumor. The temporal parameters of the mitotic cycle of the adenocarcinoma 755 were determined from the change in the percentage of labeled mitoses after a single injection of thymidine-^{3}H [10]. The isotope was injected into mice of the experimental and control groups intraperitoneally in a dose of 1-2 μCi/g body weight (specific activity 12.8 Ci/mmole). The mice were killed every 2-4 h between 1 and 28 h after injection of thymidine-^{3}H. Isolation of the tumor and preparation of films from the cell suspension were carried out by Puck's method [8]. The proliferative pool in the adenocarcinoma 755 was calculated by the method of comparing the observed and expected labeling indices (TI/LI) and also by determining the number of labeled cells after repeated injections of thymidine-^{3}H [4, 8]. For this purpose mice of the experimental and control groups were injected with isotope in a dose of 20-25 μCi seven times at 4-hourly intervals for 24 h. The cell loss factor in adenocarcinoma 755 was determined by the equa-

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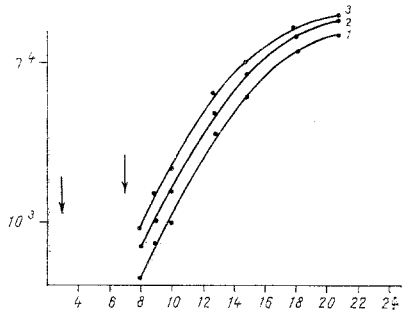

Fig. 1

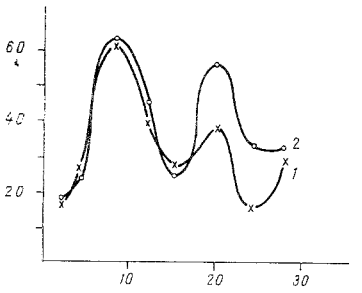

Fig. 2

Fig. 1. Growth curves of adenocarcinoma 755 in C57BL mice under the influence of methylcobalamine. Abscissa, times after transplantation of tumor (in days); ordinate, mean volume of tumor (in mm^3). Arrows indicate time of injection of methylcobalamine. 1) Control; 2) methylcobalamine (10 μg/kg × 2); 3) methylcobalamine (10 μg/kg × 5).

Fig. 2. Changes in number of labeled mitoses in mouse adenocarcinoma 755 cells at different times after a single injection of thymidine-^3H. Abscissa, time after injection of thymidine-^3H (in h); ordinate, number of labeled mitoses (in %). 1) Tumor on 8th day after transplantation; 2) after injection of methylcobalamine (10 μg/kg × 2, on the 3rd and 7th days of tumor growth).

TABLE 1. Effect of Methylcobalamine on Kinetics of Proliferation of Adenocarcinoma 755 Cells (M±m)

Group of mice	Observed labeling index (TI), %		Expected labeling index (LI), %	Ratio between observed and expected labeling indices (TI/LI)	Tumor volume doubling time, days		Cell loss (φ), %
	1 h after a single injection of thymidine-^3H	24 h after repeated injection of thymidine-^3H			T_d	T_p	
Experimental	29,6±1,0	56,9±2,1	38,2	0,7	1,2	0,7	0,4
Control	20,6±0,6	42,8±1,3		0,5	1,0	0,9	0,1
P	<0,001	<0,05					

Legend. 1) Mice of the experimental group received methylcobalamine twice in a dose of 10 mg/kg each time. 2) Labeling index determined by analysis of 5000 cells in 5-10 mice of experimental and control groups.

tion: $\varphi = 1-(T_p/T_d)$, allowing for the actual (T_d) and potential (T_p) tumor volume doubling time [9]. Autoradiographs were compared by the standard method [2]. The significance of the difference between values obtained for mice of the experimental and control groups was calculated by Student's t-test.

EXPERIMENTAL RESULTS

Growth of mammary gland adenocarcinoma 755 obeys an exponential law [6-8]. In the present investigations the character of the growth curve of adenocarcinoma 755 after injection of methylcobalamine did not change significantly, but the rate of growth of the tumor increased considerably under these conditions (Fig. 1). Stimulation of growth of adenocarcinoma 755 was most marked in the period of administration of methylcobalamine, and a significant difference in the size of the tumors was observed in the mice of the experimental and control groups during the 7 or 8 days after injection of the coenzyme. Later, growth of the tumor slowed down and its volume on the 21st day after transplantation did not differ significantly.

To assess the action of methylcobalamine on growth of mouse adenocarcinoma 755, the basic parameters of cell proliferation were determined. Calculations showed that the duration of the mitotic cycle (T_c) of the adenocarcinoma 755 cells on the 8th day of tumor growth was 12-14 h (Fig. 2). The duration of DNA synthesis (t_s) in the tumor cells under the circumstances was 6 h. The minimal duration of the postsynthetic period (t_{g_2}) did not exceed 2 h, and the total duration of the presynthetic period (tg_1) and of mitosis (t_m) was 4 h. These temporal parameters for the mitotic cycle of adenocarcinoma 755 cells are basically in agreement with data

in the literature [7, 8]. Under the influence of methylcobalamine the duration of the mitotic cycle and its separate periods in adenocarcinoma 755 cells did not change significantly (Fig. 2). Meanwhile, the number of proliferating cells in tumors of mice of the experimental group increased significantly compared with the control animals (Table 1). The size of the proliferative pool, calculated by comparing the observed and expected labeling indices, was 0.7 and 0.5 for adenocarcinoma 755 of mice of the experimental and control groups respectively. The expected labeling index in tumors determined on the basis of the duration of the mitotic cell cycle, was the same in the mice of the various groups. The observed labeling index 1 h after a single injection of thymidine-^{3}H in the adenocarcinoma 755 of mice of the experimental group was 1.4 times higher than that for the control animals. The significant increase in the proliferative pool in the tumor under the influence of methylcobalamine was also confirmed by the results of repeated injection of thymidine-^{3}H. They showed that the proliferative pool in adenocarcinoma 755 of the experimental mice was $56.9 \pm 2.1\%$, but only $42.8 \pm 1.3\%$ in the tumors in the control mice.

The cell loss factor (φ) in adenocarcinoma 755 on the 8th day of growth, it will be noted, was minimal and increased a little under the influence of methylcobalamine (Table 1).

Acceleration of growth of mouse adenocarcinoma 755 under the influence of methylcobalamine was thus due mainly to enlargement of the proliferative pool, while the temporal parameters of the mitotic cell cycle remained stable.

Selectivity of action of certain antitumor agents is known to be enhanced as a result of an increase in the number of proliferating cells in the tumor [5]. There is thus a real possibility of increasing the sensitivity of tumor cells by means of methylcobalamine to the inhibitory action of certain cycle-specific substances, notably methotrexate. In previous investigations the maximal effect of combined administration of methylcobalamine and methotrexate was observed in the late stage of growth of adenocarcinoma 755, when methotrexate alone has virtually no therapeutic action [1, 3]. With an increase in the total dose of methylcobalamine the antitumor action of methotrexate is considerably potentiated, and if the two drugs are administered simultaneously, the therapeutic effect continues despite a much smaller dose of methotrexate.

The increase in the antitumor activity of methotrexate during combined administration with methylcobalamine is largely due to an increase in the number of proliferating cells in the adenocarcinoma 755. Under these conditions methotrexate evidently inhibits DNA synthesis in a significantly larger subpopulation of cells in the S phase, which are most sensitive to its action. The intensity of assimilation of cobalamines by the tumor and the possibility that their transport into the cells may be blocked by means of a methylcobalamine antagonist are also of great importance in relation to combined treatment. The results of all the writers' investigations are serving as the basis for elaboration of the most effective use of cobalamine derivatives in the chemotherapy of malignant neoplasms.

LITERATURE CITED

1. F. G. Arsenyan, N. V. Myasishcheva, Z. P. Sof'ina, et al., Khim.-Farm. Zh., No. 10, 49 (1978).
2. O. I. Epifanova, V. V. Terskikh, and A. Ya. Zakharov, Autoradiography [in Russian], Moscow (1977).
3. Z. P. Sof'ina, N. V. Myasishcheva, F. G. Arsenyan, et al., Vestn. Akad. Med. Nauk SSSR, No. 1, 72 (1979).
4. O. S. Frankfurt, The Cell Cycle in Tumors [in Russian], Moscow (1975).
5. O. S. Frankfurt, Cellular Mechanisms of Tumor Chemotherapy [in Russian], Moscow (1976).
6. N. M. Émanuél', The Kinetics of Experimental Tumor Processes [in Russian], Moscow (1977).
7. W. R. Laster, J. G. Mayo, L. Simpson-Herren, et al., Cancer Chemother. Rep., 53, 169 (1969).
8. L. Simpson-Herren and H. H. Lloyd, Cancer Chemother. Rep., 54, 143 (1970).
9. G. G. Steel, Cell Tissue Kinet., 1, 193 (1968).
10. H. Quastler and F. G. Sherman, Exp. Cell Res., 17, 420 (1959).