METHIONINE SYNTHETASE ACTIVITY IN TUMOR CELLS IN MICE AFTER INJECTION OF METHYLCOBALAMINE AND ITS ANALOG

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Methionine synthetase is one of the key enzymes which control the formation of active forms of tetrahydrofolate that are essential for the intensive metabolism of C_1-fragments in proliferating cells [7, 10]. The coenzyme of methionine synthetase is methylcobalamine, which activates the transfer of the methyl group from methyltetrahydrofolic acid to homocysteine, with the formation of methionine and tetrahydrofolate. Methylcobalamine has been shown to have a stimulating action on proliferation of normal cells and of certain transplantable animal tumors [1, 2]. It has also been shown that growth of certain bacteria, cultures of human embryonic fibroblasts, and certain transplantable tumors in vivo can be inhibited by means of methylcobalamine analogs [3, 4]. However, the biochemical mechanisms by means of which methylcobalamine and its analogs influence processes of cell proliferation have not yet been adequately studied [9, 14]. No data have yet been obtained on the activity of cobalamine-dependent methionine synthetase in tumor cells during the action of methylcobalamine and its analogs in vivo.

The object of this investigation was to study methionine synthetase activity and the proliferative pool of mouse mammary gland adenocarcinoma (Ca-755) cells after injection of methylcobalamine and its analog, methylcobalamine chloropalladate, into animals.

EXPERIMENTAL METHOD

The tumor was transplanted into female C57BL mice by subcutaneous injection of a suspension of tumor cells ($1 \cdot 10^6$ cells in 0.5 ml of medium 199 per mouse). Methylcobalamine (MeCbl) was injected intramuscularly in a dose of 10 μg/kg body weight on the 3rd and 5th days after transplantation of the tumor. Methylcobalamine chloropalladate (MeCbl·$PdCl_3$) was administered per os to the animals of the other group in 2% starch suspension in a dose of 250 mg/kg on the 2nd and 6th days after transplantation of the tumor. The compound was synthesized in the Laboratory of Thio-Organic Compounds of the "Vitaminy" Scientific Production Combine [6]. Animals with tumors not receiving the preparations served as the control. On the 8th day after transplantation, methionine synthetase activity was measured and the proliferative pool of tumor cells estimated. Methionine synthetase activity was determined in extracts of tumor cells by measuring the quantity of labeled methionine formed by the enzyme from methyltetrahydrofolate-5-^{14}C and homocysteine. Methionine-^{14}C was separated from labeled tetrahydrofolate on a Dowex 1 × 8 Cl^- column (3 × 0.5 cm) with elution by water. Activity of the enzyme was expressed in nanomoles methionine formed per hour per milligram protein [11, 12]. To measure the initial quantity of active (bound with MeCbl) holoenzyme in extracts of tumor cells, either no cobalamine coenzymes were added to the reaction mixture or cyanocobalamine (CNCbl), 50 μM, was added. The total quantity of enzyme (apo- and holoforms) was determined after addition of MeCbl ($1 \cdot 10^{-5}$M) to the reaction mixture. The proliferative pool in

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TABLE 1. Effect of Methylcobalamine and Its Chloropalladate on Methionine Synthetase Activity and on the Proliferative Pool in Ca-755 Cells

Experimental conditions	Methionine synthetase				Proliferative pool	
	coenzymes in reaction mixture	number of animals	activity, nmoles methionine/mg protein/h	quantity of holoenzyme, %	ratio between observed and expected labeling indices	labeling index after repeated injections of thymidine-^{3}H
Control	O	3	5,0	48	0,5	42,8±1,3
	CNCbl	13	5,8	56	—	—
	MeCbl	6	10,3	100	—	—
Injection of MeCbl	CNCbl	7	10,5	—	0,7	56,9±2,1
Administration of $MeCbl \cdot PdCl_3$	CNCbl	10	6,7	43	—	48,1±1,5
	MeCbl	3	15,5	100	—	—

the tumor was estimated from the number of labeled cells after repeated injection of thymidine-^{3}H (1-2 μCi/g 7 times in the course of 24 h) into the animals, and also by comparing the observed and expected labeling indices [5, 13]. The labeling index was determined by analysis of 5000-10,000 cells on autoradiographs obtained from 10 animals of each test group.

EXPERIMENTAL RESULTS

The results of comparison of methionine synthetase activity, the quantity of holoenzymes, and the proliferative pool of adenocarcinoma Ca-755 cells in different groups of animals are given in Table 1. The fact that methionine synthetase activity in tumor extracts from control animals could be doubled after the addition of MeCbl to the reaction mixture is evidence that a high proportion of the methionine synthetase was present in Ca-755 cells only in the potentially active apo-form.

Methionine synthetase activity measured in the presence of CNCbl in tumors of animals into which MeCbl was injected in vivo was twice as high as the corresponding control values and was equal to the reaction velocity in the control in the presence of MeCbl. This can be interpreted as evidence that after injection of MeCbl into animals the whole of the enzyme in the tumor is in the active holo-form. The increase in methionine synthetase activity in the Ca-755 cells after injection of MeCbl into the animals coincided in time with a 50% increase in the size of the proliferative pool of the tumor. It was shown previously that MeCbl has a stimulating action on growth of Ca-755 [1]. If activation of methionine synthetase is of essential importance for the stimulation of cell proliferation, a decrease in activity of the enzyme may inhibit tumor growth. In fact, the methylcobalamine analog $MeCbl \cdot PdCl_3$ retarded growth of the Ca-755 tumor by 80-90% by the 8th day after transplantation [4]. However, measurements of methionine synthetase activity in the tumors in this group of animals showed that in the presence of CNCbl the reaction velocity was not significantly changed. Addition of MeCbl to the reaction mixture considerably increased the reaction velocity. Calculation showed that the quantity of holoenzyme in the extracts under these circumstances was about 40%, i.e., there was a tendency for the quantity of the form of enzyme bound with MeCbl to diminish. The size of the proliferative pool in tumors in this group of animals on the 8th day after transplantation did not differ significantly from the control (Table 1). According to data obtained on cultures of human lymphoblasts, methionine synthetase activity is maximal at the beginning of the logarithmic phase (log-phase) of cell growth and then falls rapidly to its initial value [8]. In the present experiments the analog evidently retarded tumor growth in the early log-phase actually during the period of its administration to the animals. In later stages the inhibitory effect was reduced and by the 14th day it amounted to only 59% compared with the control [1].

Only half of the total quantity of the test enzyme in Ca-755 cells is thus in the active holo-form. After administration of MeCbl to animals, methionine synthetase activity rises simultaneously with an increase in the pool of proliferating cells in the tumor. Significant inhibition of tumor growth as a result of administration of methylcobalamine chloropalladate to animals is observed against the background of some decrease in the quantity of methionine synthetase in the bound form with MeCbl. Inhibition of tumor growth thus observed is probably due to competitive interaction between the test compounds in vivo.

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