INFLUENCE OF METHYLCOBALAMIN ON THE ANTINEOPLASTIC ACTIVITY OF METHOTREXATE

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UDC 615,277.3.015.2:615.355:577. 152.611'.133

One of the possible ways of increasing the selectivity of the action of chemotherapeutic substances on tumor cells is the combined use of preparations, taking the peculiarities of the mechanism of their action into account. A new trend in this field is the use of cobalamin derivatives in combination with definite antineoplastic preparations.

The special significance of methylcobalamin was first noted in the case of impaired cobalamin metabolism in leukemia patients. An analysis of the functional activity of cobalamin coenzymes in the organism, in comparison with the effectiveness of combined cytostatic therapy, has shown that the clinical course of the process in acute leukemia with an increased content of hydroxy- and methylcobalamins in the blood is less favorable [1]. The results obtained were evidence of the important role of methylcobalamin in metabolic processes as a coenzyme of methionine synthetase (EC 2.1.1.13)—a key link in the control of the synchronized action of cobalamins in compounds of folic acid in processes of cell proliferation [1-2].

A study of the morphofunctional state of the hemopoietic system of animals under conditions of intensive cobalamin metabolism in the organism confirmed the fact that at a high concentration of cobalamin coenzymes, the rate of proliferation of cells of the hemopoietic tissue increases. In the spleens of healthy mice, in the case of prolonged administration of methylcobalamin, hyperplasia of the lymphoid elements, an increase in the number of DNA-synthesizing cells, and an increase in their mitotic index were noted. The stability of the periods of the mitotic cycle of spleen lymphocytes in the presence of an increase in the size of the proliferative

TABLE 1. Stimulating Effects of Methylcobalamin on the Growth of Transplantable Tumors of Mice

Tumor	Line of mice	Dose of preparation, μg/kg	Increase in tumor volume after administration of methylcobalamin, % of control		
			7-8th day*	12-14th day*	21st day*
Ca-755	BDF_1	10	—	—	+10†
	$C_{57}BL$	10	+75	+40†	+77
	F_1	500	+45	+15†	+30
	F_1	500	+77	+29†	+52
AKATOL	BALB/c	10	+126	+37	+33
RShM-5	CBA	10	+47	0	0
Sarcoma 37	SHK	500	+57	0	0

*Period after transplantation of tumor.
†$P > 0.05$, in all remaining cases $P < 0.05$.
Note. Here and in Table 2: the preparation was administered on the second and sixth days after transplantation of the tumor. A "plus" sign denotes stimulation of tumor growth.

Oncological Scientific Center of the Academy of Medical Sciences of the USSR. Scientific-Industrial Vitamin Combine, Moscow. Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 12, No. 10, pp. 49-54, October, 1978. Original article submitted April 3, 1978.

0091-150X/78/1210-1299 $07.50

TABLE 2. Results of Combined Action of Methylcobalamin and Methotrexate on the Growth of Ca-755 (BDF_1)

Preparations	Dose of preparation	Inhibition of tumor growth* after course of administration of preparations, % of control		Increase in lifetime of animals, %
		1st-2nd day†	7-8th day†	
Methotrexate	10 mg/kg	94	51	19‡
Methylcobalamin	10 μg/kg	+ 180	+ 65	0
Methylcobalamin + methotrexate	10 mg/kg (simultaneously) 10 μg/kg	94	76	60
Methylcobalamin + methotrexate	10 μg/kg (methotrexate was administrated 6 h after methylcobalamin)	+ 36	+ 62	21‡

*Average results of five series of experiments.
†Period after transplantation of tumor.
‡$P > 0.05$; in all remaining cases $P < 0.05$. In the case of combined influence, the results obtained were evaluated relative to methotrexate.

pool made it possible to conclude that the intensified proliferation of hemopoietic cells under these conditions is due to an increase in the number of cells entering the mitotic cycle [3-5]. Further experimental investigations revealed the active role of methylcobalamin not only in processes of proliferation of cells of the hemopoietic tissue. An analogous influence on proliferative activity (an increase in the fraction of cells labeled with [^{3}H]thymidine and an increase in the mitotic index) has also been detected in various periods of culturing of embryonic human fibroblasts in media with a high methylcobalamin concentration [6-7].

In view of the fact that normal cells of adult animals, embryonic and tumor cells differ in their ability to respond to the inducing influence of cobalamins, it was necessary to evaluate the action of methylcobalamin on processes of growth of various types of tumors.

The stimulating influence of cyanocobalamin on the growth and development of certain transplantable (sarcoma 45, Guerin carcinoma, Walker carcinosarcoma, sarcoma 180, Lewis sarcoma, etc.) and induced tumors is evidently due to its conversion to cobalamin coenzymes in the animal organism. Methylcobalamin and adenosylcobalamin have been detected in spleen cells of mice with La Leukemia, as well as in leukemia L-1210 and Ehrlich's ascites carcinoma cells [8-10].

The aggregate of clinico-experimental data thus determined the advisability of the search for effective antagonists of cobalamins for the blocking of certain cobalamin-dependent reactions. In view of the activating influence of methylcobalamin on methionine synthetase and the increase in the total pool of tetrahydrofolic acid (THFA) in the cells, regardless of the folate reductase system, the greatest attention is attracted by antagonists of methylcobalamin [11-13]. In our investigations using methylcobalamin antagonists to lower the methionine synthetase activity, we succeeded in slowing down the processes of growth of bacterial and embryonic cells, as well as certain types of tumors [6, 14]. It was also shown that the antineoplastic activity of methotrexate – a specific inhibitor of folate reductase – increases when it is used in combination with methionine synthetase inhibitors [14].

In addition, there is still another possibility of enhancing the antineoplastic activity of methotrexate with cobalamins. The prerequisite for this means of combined influence with methotrexate was experimental data showing the ability of cobalamins to stimulate processes of proliferation and to increase the number of DNA-synthesizing cells, most sensitive to methotrexate, in the population [15, 4].

The present communication presents the results of the combined action of methylcobalamin and methotrexate on various transplantable tumors in animals.

EXPERIMENTAL

The experiments were conducted on mice of the $C_{57}BL$, CBA, and BALB/c lines, the hybrids BDF_1-($C_{57}BL \times DBA/2$), $F_1(C_{58}BL \times CBA)$ and SHK mice, obtained from the nursery of the Academy of Medical Sciences of the USSR. In the experiments we used 420 mice, weighing 20-25 g.

The action of methylcobalamin was studied on solid tumors: adenocarcinoma of the mammary gland (Ca-755), cancer of the cervix (RShM-5), adenocarcinoma of the intestine (AKATOL), sarcoma 37, as well as on leukemia L-1210, according to the procedure used in the laboratory [16, 14].

Methylcobalamin (CH_3Cbl), synthesized according to the method of [17], was injected intramuscularly in doses of 10 and 500 μg/kg twice at 96-h intervals or 500 μg/kg daily for five days.

Methotrexate (from Lederle) was used in a dose of 10 mg/kg intraperitoneally twice at a 96-h interval.

In part of the experiments, methylcobalamin chloropalladate ($CH_3Cbl \cdot PdCl_3$; I) and dibromide-4-[[[[[[1-methylpyridino-4-amino]phenyl]amino]carbonyl]phenyl]amino]-6-amino-1-methylquinoline (NSC-176319; II), which we obtained from the National Cancer Institute of the United States according to the program of cooperation between the USSR and the US in the field of chemotherapy of tumors [18], were used as methionine synthetase inhibitors.

Complex I, synthesized at the All-Union Vitamin Scientific Research Institute [19], was administered perorally in a dose of 250 mg/kg; the quinolinium derivative II was administered intraperitoneally in a dose of 5 mg/kg twice at a 96-h interval.

The treatment of the animals was begun 48 h after transplantation of the tumor. The antineoplastic effect was estimated directly after the end of the course of therapy and at various periods over the subsequent life of the animals.

The criteria of effectiveness were the percent inhibition of tumor growth, calculated according to its volume, and the increase in the lifetime of the animals. The data obtained were subjected to statistical treatment according to the Student method.

RESULTS AND DISCUSSION

From the data that we obtained it follows that methylcobalamin substantially stimulates the growth of transplantable tumors: Ca-755, AKATOL, and to a lesser degree RShM-5 and sarcoma 37 (Table 1).

The intensity of tumor growth depended on the line of experimental animals, the frequency of administration, and the concentration of methylcobalamin. The greatest stimulating effect on growth of the tumor Ca-755 was noted in the case of two administrations of the preparation in a dose of 10 μg/kg after transplantation of the tumor into the hybrids BDF_1 (+180%), and to a lesser degree for mice of the pure line $C_{57}BL$ (+75%). In F_1 hybrids, a substantial intensification of tumor growth was detected in the case of five administrations of methylcobalamin in a dose of 500 μg/kg. The stimulation of the growth of Ca-755 and AKATOL was followed for a period of two to three weeks, whereas in mice with sarcoma 37 and RShM-5, it was noted only directly after the end of the course of administration of the preparation. In mice of the pure line ($C_{57}BL$), intensified tumor growth was observed for a longer period (2-3 weeks after transplantation of the tumor) than in hybrids. For precisely this reason, in subsequent investigations of the action of methylcobalamin and its analogs on the cell kinetics of Ca-755, we used mice of the $C_{57}BL$ line.

In the case of simultaneous administration of methotrexate and methylcobalamin, an intensification of their inhibiting effect on tumor growth was observed (L-1210, Ca-755, RShM-5). The lifetime of animals with leukemia L-1210 was increased by 78% in this case, whereas in the case of isolated administration of methotrexate the increase was only 55%. The most rapid results were obtained for adenocarcinoma of the mammary gland (Table 2). In this case the combination of methotrexate with methylcobalamin increased the lifetime of the animals by 60%, which was three times as great as the effect of methotrexate alone. On the 8th to 14th days after the end of the combined course of therapy with methylcobalamin and methotrexate, the inhibition of tumor growth was 76-40%, respectively, whereas methotrexate alone had practically no activity at the same periods (51-0%).

It is known that as solid tumors grow, the number of cells in the resting phase in them increases substantially, and the sensitivity of the tumors to cyclospecific preparations decreases appreciably [20]. Evidently the sensitivity of the tumor to methotrexate can be substantially increased by administering methylcobalamin,

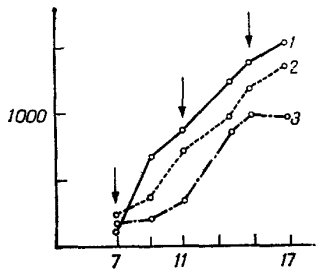

Fig. 1

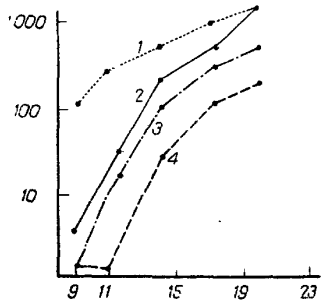

Fig. 2

Fig. 1. Combined action of methylcobalamin and methotrexate on growth of RShM-5. Along x-axis: period after administration of preparation (in days); along y-axis: average volume of tumor (in mm^3). The arrows indicate the time of administration of the preparations. 1) Control; 2) methotrexate; 3) methylcobalamin + methotrexate.

Fig. 2. Combined action of methylcobalamin, methionine synthetase inhibitors, and methotrexate on growth of Ca-755. Along x-axis: periods after administration of preparations (in days); along y-axis: average volume of tumor (in mm^3). 1) Control; 2) methotrexate; 3) CH_3Cbl + NSC-176319 + $CH_3Cbl \cdot PdCl_3$ + methotrexate; 4) NSC-176319 + $CH_3Cbl \cdot PdCl_3$ + methotrexate.

which increases the total pool of DNA-synthesizing cells. To test this hypothesis we used transplantable mouse cancer of the cervix (RShM-5). This tumor is characterized by slow growth, which makes it possible to administer a longer course of therapy and to evaluate the antineoplastic effect in long-term periods after transplantation. The experimental results confirmed our hypothesis. The average volume of the tumor in the control group of animals on the 11th day after transplantation of RShM-5 exceeded the initial volume, when treatment of the animals was begun (7th day after transplantation), by 5.2-fold. In animals that received only methotrexate, the volume of the tumor at the same periods was increased 3.5-fold, and in the case of joint administration with methylcobalamin, there was only a 1.7-fold increase. Repeated combined administrations of the preparations (each four days) led to an inhibition of tumor growth in longer term periods as well. Thus, on the 17th day after transplantation, the average volume of the tumors in the control group exceeded the original volume by 22.7-fold, in the group of animals treated with methotrexate by 12.7-fold, and in the group of mice that received methylcobalamin and methotrexate by 7.6-fold (Fig. 1).

The interval between administration of methylcobalamin and methotrexate is of vital importance. According to the data obtained, with increasing time between administrations of the preparations, a partial or total loss of activity of methotrexate is noted, and in certain cases even the appearance of an effect of stimulation (see Table 2). Thus, for example, the inhibition of growth of Ca-755 on the 7th day after the end of treatment with methotrexate was 69%. And yet, when methylcobalamin was preliminarily administered (6 h before the use of methotrexate), a total loss of activity of methotrexate was observed. The weakening of the antineoplastic activity of methotrexate is especially pronounced in the hybrids BDF_1. As was shown , precisely in mice of this line, methylcobalamin induced the greatest stimulation of tumor growth. In F_1 hybrids with the absence of a stimulating effect, in the case of its isolated use, the combined influence did not lead to any weakening of the methotrexate activity. An appreciable decrease in the antineoplastic activity of methotrexate after preliminary administration of methylcobalamin is evidently due to activation of the cobalamin-dependent methionine synthetase system and an increase in the total pool of the TGFA of the cells. This is confirmed by the results of the combined action of methotrexate and inhibitors of methionine synthetase against a background of preliminarily administered methylcobalamin (Fig. 2). The joint influence of methylcobalamin chloropalladate, the quinolinium derivative, and methotrexate substantially exceeds the activity of a combination of the same preparations with methylcobalamin. Thus, the inhibition of growth of Ca-755 on the 14th day after the end of therapy of mice that received methotrexate and complexes II and I was 85%, whereas in the case of combined influence of the three inhibitors with methylcobalamin it was only 61%. The increase in the lifetime of the animals in these groups was 30% ($P < 0.05$) and 15% ($P > 0.05$), respectively.

Thus, in our investigations the stimulating action of methylcobalamin on the growth of certain solid tumors in mice was demonstrated for the first time. The results of our investigations permit an explanation of the decrease in the therapeutic effect of a number of alkylating preparations (sarcolysin, thioTEPA, embitol, and novembitol) in the case of their simultaneous use with cyanocobalamin [21-23]. The stimulating effect of methylcobalamin on solid tumors is clearly correlated with recent investigations, in which a significant increase in the methylcobalamin content in the rat liver was revealed after the administration of a chemical carcinogen and in certain transplantable Morris hepatomas [24]. It is important to note that the frequency of development of hemoblastoses of mice and of the simultaneous influence of methylcobalamin with endogenous blastomogens also increases significantly [25]. The aggregate of the indicated experimental data thereby confirms the involvement of methylcobalamin in processes of proliferation of tumor cells of various histogenesis.

In discussing the mechanism of the combined action of methylcobalamin with methotrexate, in our opinion, we should consider two possible aspects. In view of the fact that cobalamins promote the entry of the basic transport form of folic acid (methyl-THFA) into cells, and there is a common pathway of active transport of methyl-THFA and methotrexate into cells [26, 27], it can be assumed that methylcobalamin also influences the transport of methotrexate. At present there are no data in the literature on the mechanism of the penetration of methotrexate into Ca-755 cells. However, under our conditions of influence, at a physiological level of methyl-THFA in the blood of the animals and the therapeutic concentration of methotrexate, evidently the possibility of facilitated penetration of the latter into the tumor cells is realistic.

Vitally important factors in the combined influence are activation of the methionine synthetase reaction and an increase in the number of DNA-synthesizing tumor cells, i.e., those most sensitive to methotrexate, under the influence of methylcobalamin. This may play a deciding role in the increase in the antineoplastic activity of methotrexate when it is administered simultaneously with methylcobalamin. The data that we obtained at present on the study of the cell kinetics of Ca-755 under the influence of methylcobalamin confirm this premise.

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