EXPERIMENTAL STUDY OF THE COMBINED ACTION OF 5-FLUOROURACIL AND CYCLOPHOSPHAMIDE ON GROWTH OF ZAJDELA'S HEPATOMA AND ON NUCLEIC ACID METABOLISM

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A combination of two or more antitumor preparations is widely used in the chemotherapy of malignant tumors. However, the development of schemes for therapeutic application of different combinations of therapeutic substances has proceeded empirically. The elucidation of the mechanism of the combined action of preparations at the molecular level could make a great contribution to rational approaches in this field. In particular, a combination of 5-fluorouracil (5FU) and cyclophosphamide (CP) is used in practice. However, the mechanism of action of this combination is unknown.

Considering existing views on the way in which the two substances intervene in nucleic acid metabolism, an attempt was made in the investigation described below to study the action of these compounds in different combinations on tumor growth and nucleic acid synthesis in tumor cells.

EXPERIMENTAL METHOD

Male rats weighing 100-150 g with a transplanted Zajdela's hepatoma were used. Treatment began 24 h after intraperitoneal injection of a suspension of tumor cells. The compounds were injected intramuscularly once a day for 6 days. The animals were decapitated 24 h after the end of treatment.

The experiments were divided into two series, each of which included six groups of animals: 1) control; 2) treatment with 5FU only, 3) treatment with CP only, 4) consecutive treatment with 5FU followed by CP; consecutive treatment with CP followed by 5FU; 6) treatment with 5FU and CP simultaneously. The antitumor activity of the treatment was judged from the weight of the tumor cells obtained after centrifugation of ascites fluid for 10 min at 1400g. The cells were incubated as described previously [4]. The toxicity of the preparations was judged by the number of myelokaryocytes in the bone marrow of the rats on the day the experiment ended.

The rate of nucleic acid synthesis was measured from the incorporation of labeled precursors; ^{3}H-uridine into total RNA, ^{3}H-thymidine and ^{3}H-uridine into DNA. Total RNA was isolated by the phenolic method [5] in our own modification. The cells were suspended in 0.1 M solution of Tris-HCl buffer, pH 7.0, in the ratio of 1:19, an equal volume of cold phenol was added, and the mixture was homogenized. A solution of sodium dodecylsulfate was then added to a final concentration of 0.5% and the mixture was shaken for 5 min in the cold and for 15 min at 75°C. Further treatment of the samples was carried out without modification. Analysis and preparative isolation of the fraction of total RNA were carried out by electrophoresis in polyacrylamide gel in the writers' modification [3]. The concentration of acrylamide was reduced to 2.32% and, at the same time, the concentrations of bisacrylamide, ammonium persulfate, and TEMED were increased to 0.15%. A buffer solution of the following composition was used for fractionation: 28 g Tris (hydroxyethylaminomethane), 24 g monosodium hydrogen phosphate, 40 g disodium hydrogen phosphate, water to one liter (main solution); diluted 1:30 before use. DNA was extracted from the interphase after isolation of total RNA [1]. To remove

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TABLE 1. Antitumor Activity and Intensity of Nucleic Acid Synthesis during Treatment of Zajdela's Hepatoma by 5FU and CP in Various Combinations

Group of animals	Preparations used for treatment	Days of administration	Dose of prepn., mg/kg body wt.	Weight of tumor cells, g	Specific radioactivity, cpm/mg					
					DNA ($\cdot 10^{-3}$)		RNA ($\cdot 10^{-3}$)			
					^{3}H-uridine	^{3}H-thymidine	4S	18S	28S	32S—45S
1	Control	—	—	4,7±1,4	107±12	34±8	590±38	517±33	510±32	540±46
2	5-FU	1—6	10	4,3±2,0	100±19	21±2	886±28*	459±35	293±38*	287±42*
3	CP	1—6	5	4,7±1,6	79±14	16±2*	767±40*	509±58	370±74*	524±57
4	5-FU	1—3	10	0,9±0,3*	57±5,4*	9±1*	178±17*	137±13*	92±23*	136±24*
	CP	4—6	5							
5	CP	1—3	5	2,5±1,1	38±5*	6±1*	591±41	129±24*	155±26*	415±72
	5-FU	4—6	10							
	CP	1—6	5	1,9±1,0	19±1*	6±0,5*	369±44*	165±5*	81±18*	123±24*
6	5-FU		10							

*Here and in Table 2, difference from control significant ($P < 0.05$).

TABLE 2. Antitumor Activity, Medullary Hematopoiesis, and Intensity of Nucleic Acid Synthesis during Treatment of Zajdela's Hematoma by 5FU and CP by Different Schemes

Group of animals	Preparations used for treatment	Days of administration	Dose of preparation, mg/kg body weight	Weight of tumor cells, g	Myelokaryocytes/mm^3	Specific radioactivity, cpm/mg					
						DNA ($\cdot 10^{-3}$)		total RNA ($\cdot 10^{-3}$)			
						^{3}H-thymidine	^{3}H-uridine	4S	18S	28S	32S—45S
1	Control	—	—	7,1±0,6	1441600±12400	107±12	34±7	590±38	517±38	510±32	540±46
2	5-FU	1—6	20	1,8±0,3*	728000*±194000	51±10*	24±3,5	295±33*	226±27*	124±10*	185±55*
3	CP	1—6	10	2,0±0,4*	790000*±42000	26±6*	3,4±1,6*	388±27	161±30*	99±11*	102±7*
4	5-FU	1—3	20	3,0±0,7*	1228000±150000	24±8*	4,6±1,2*	170±20*	140±28*	45±6*	85±12*
	CP	4—6	10								
5	CP	1—3	10	2,5±0,6*	739600*±56000	23±6*	4,8±1,0*	420±60	240±36*	207±50*	260±40*
	5-FU	4—6	20								
6	CP + 5-FU	1—6	10 20	1,9±0,3*	391000*±83000	18±4*	3,6±1,0*	308±48*	100±22*	80±16*	97±28*

possible traces of RNA as impurities, the resulting DNA was treated with ribonuclease [2].

EXPERIMENTAL RESULTS

It will be clear from Table 1 that administration of 5FU in a dose of 10 mg/kg and CP in a dose of 5 mg/kg did not inhibit tumor growth. Incorporation of ^{3}H-thymidine into DNA in the tumor cells was only slightly inhibited. Meanwhile, an increase was observed in the rate of incorporation of ^{3}H-uridine into the low-polymer fraction of total RNA, and synthesis of 28S ribosomal RNA was considerably inhibited. However, the successive administration of these drugs by the group 4 scheme (3 days 5FU, next 3 days CP) caused sharp inhibition of tumor growth. The rate of synthesis of all fractions of total RNA and of incorporation of both precursors into DNA was sharply reduced in the tumor cells.

A change in the order of administration of the compounds to that in group 5 led to a reduction in the antitumor effect, although incorporation of both precursors into DNA was inhibited more sharply than in the tumor cells in the animals of group 4. The intensity of synthesis of low-polymer fractions of total RNA (4S) was unchanged compared with the control.

Simultaneous administration of the compounds (group 6) also led to inhibition of DNA synthesis; the inhibition, moreover, was significantly greater than in group 4. Synthesis of ribosomal RNA (18S and 28S) was inhibited just as effectively as in the tumor cells of animals of groups 4 and 5; synthesis of low-polymer (4S) RNA was significantly less in this case than in group 4.

It can be tentatively suggested that the antitumor action of the compounds was due to their interaction with DNA and subsequent disturbance of the functioning of DNA as template for RNA synthesis. The antitumor effect, to judge from these results, was connected with damage to synthesis of all fractions of total RNA.

The results of the experiments of series II are given in Table 2: In this series the compounds were given in accordance with the same scheme but in doses for which treatment by each compound seprately gave a good antitumor effect. Combined administration of 5FU and CP in this case did not cause any further inhibition of tumor growth than that obtained by the administration of these compounds separately. However, when treatment was started with 5FU and ended with CP, no decrease in the number of myelokaryocytes was observed in the animals' bone marrow. Simultaneous administration of the compounds was most toxic. Depending on the scheme of administration of the compounds, the most characteristic changes affected the rate of synthesis of the low-polymer fraction (4S) of total RNA. Synthesis of low-polymer fraction of total RNA was not blocked in the tumor cells of animals receiving CP alone or whose treatment began with CP. The effectiveness of inhibition of synthesis of the ribosomal fractions of RNA (18S and 28S) was unchanged whatever scheme of administration of the compounds was used.

The results of these experiments show that treatment of rats with Zajdela's ascites hepatoma with a combination of 5FU and CP is more effective if treatment begins with 5FU and ends with CP. In this case inhibition of tumor growth was obtained without any marked side effects on medullary hematopoiesis, together with more intensive damage to nucleic acid synthesis in the tumor cells.

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