EFFECT OF CHROMOTHERAPY ON LYMPHOCYTES AND ON HUMAN TUMOR XENOGRAFTS IN NUDE MICE

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Human tumor strains, capable of being transplanted into nude mice, have been produced at the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, and used as adequate models for, in particular, preclinical trials of antitumor preparations.

Since peripheral blood is one of the first targets for side effects of chemotherapy with alkylating agents, in order to determine the sensitivity of a number of human tumors to therapeutic action, in the investigation described below it was decided to study the number of blood cells and functional activity of peripheral blood lymphocytes in nude mice.

EXPERIMENTAL METHOD

Experiments were carried out on nude mice (based on the BALB/c line), with subcutaneously transplanted strains of human tumors: melanoma (Mel-1), two strains of lung cancer (RL-1 and RL-4), carcinoma of the stomach (RZh), carcinoma of the liver (RP), and carcinoma of the urinary bladder (RMP) [3, 4]. Six mice were used in each group. Substance No. 0.380.369 (in phase 1 of clinical study) was injected intraperitoneally into mice under optimal conditions — in a single dose of LD_{10}, 6-17 days after transplantation, when palpable tumors were present (0.1-0.6 cm^3 in volume).

Antitumor activity was assessed during tumor growth by determining the percentage of inhibition, calculated with respect to the difference in volumes of tumors in the control and treated mice, and the animals remained under observation until they died. The length of survival of the mice was used as an indicator of the toxicity of the preparation and as an additional indicator of its antitumor action.

Peripheral blood (from the caudal vein) was studied in intact nude mice (background) and mice with tumors, in groups of the control and treated animals 6 days after injection of the preparation.

The blood cells were counted and the functional state of the lymphocytes determined with respect to activity of enzymes of energy metabolism: succinate dehydrogenase (SDH) and α-glycerophosphate dehydrogenase (GPDH) [2]. Enzyme activity was determined by counting the number of formazan granules in 50 cells, after which the mean level of enzyme activity per lymphocyte (relative activity) was calculated.

The results were subjected to analysis of variance and nonparametric statistical tests [1].

EXPERIMENTAL RESULTS

By their morphological characteristics the strains studied were identical with the original human tumors [3]. The time course of growth of the tumors and the survival period of the mice with tumors in the control were characteristic for each strain. Data on sensitivity to treatment are given in Table 1.

Strain Mel-1 was obtained from a cell line. The average duration of survival of mice in the control was 60 days, by which time the volume of the tumors had reached 6 cm^3. After a single injection of the preparation inhibition of growth of the treated tumors in the course of 20 days remained at the 60% level. The strain RL-1 of adenocarcinoma of the

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TABLE 1. Inhibition of Growth of Human Tumor Strains, Absolute Number, and Relative Activity of Enzymes of Energy Metabolism in Peripheral Blood Lymphocytes of Nude Mice

Experimental conditions	% of inhibition, days after treatment		Lymphocytes, thousands		SDH, per lymphocyte		GPDH, per lymphocyte	
	10	20	before treatment	after treatment	before treatment	after treatment	before treatment	after treatment
Intact animals	—	—	6,3	—	10	—	7,8	—
Mel-1	62	66	5,9	1,6[a,b]	10,3	9,2	9,3	12,6[a,b]
RL-1		82	3,6[a]	1,1[a,b]	11,7	11,8[a,b]	12,2[a]	12,4
RL-4	63	87	3,1[a]	1,0[a,b]	15,0[a]	9,8	13,5[a]	11,2
RZh	81	54	1,7[a]	0,8[a,b]	11,6	11,1	11,8[a]	10,4
RP		87	4,5[a]	1,4[a,b]	11,4	10,3	11,6[a]	10,7
RMP	70	92	4,2[a]	1,3[a,b]	17,4[a]	6,5[a,b]	12,5[a]	10,7

Legend. a) Value statistically significant relative to background (intact mice); b) also relative to control (before treatment).

lung was derived from material obtained during an operation, whereas strain RL-4 was obtained from a cell line. The mice survived on average 67 and 43 days, respectively, in the control, and the largest volume of the tumors was 6.2 and 9.9 cm^3. Both strains were highly sensitive to the preparation, and by the 20th day inhibition exceeded 80%. RZh (an adenocarcinoma, obtained from material removed at operation) led to death of mice in the control series on average after 45 days, when the volume of the tumors was 5 cm^3. RZh was distinguished by slow growth, and for that reason the preparation was injected 17 days after transplantation, and inhibition of tumor growth amounted to 81%.

With RP (hepatocellular carcinoma from a cell line) the mean volume of the tumors was greatest (over 20 cm^3) in the control, and the mice died after 43 days. Inhibition of growth of the treated tumors was 87%. Mice with RMP (a transitional-cell carcinoma with solid structure, obtained from a cell line) lived 21-28 days in the control series, and the mean volume of the tumors was 16 cm^3. Inhibition of growth of the treated tumors was 92%.

All the strains of human tumors studied, as was expected, were thus highly sensitive to the preparation, for it was chosen for clinical trials on the basis of high activity against transplantable mouse epithelial tumors and spontaneous canine tumors. Chemotherapy lengthened the period of survival of the nude mice with RMP (by 100%) and with RP (by 80%).

Six days after a single injection of the preparation a marked decrease was found in the total leukocyte count (40-70% of the control) on account of lymphocytes. Significant lymphocytopenia also was observed in a preclinical study of the preparation on mice, rats, and dogs. It was therefore deemed particularly important to study not only the peripheral blood formula, but also the state of lymphocyte function in treated mice compared with the control.

The first point to be noted is that transplantation of the tumors caused a change in the peripheral blood parameters compared with the background values. These parameters were most stable in melanoma. Transplantation of carcinoma of the lung, liver, and urinary bladder caused a decrease of 1.5-2-fold in the absolute lymphocyte count, whereas transplantation of RZh led to a decrease of more than 3-fold in the background value, accompanied by a small increase in the number of neutrophils, which was significant in the case of RMP. Chemotherapy led to an even greater decrease in the lymphocyte count compared with the corresponding control for each strain, and the number of neutrophils was unchanged (Table 1). The number of lymphocytes fell to 1000-1600, or 27-32% of the control value in Mel-1, RL-1, RL-4, RP, and RMP. In the case of RHz, transplantation of which caused the most significant reduction in the lymphocyte count, injection of the preparation caused a smaller reduction in the lymphocyte count (up to 47% of the control) compared with the other strains, but due to the lymphocytopenia, proviked by transplantation of the tumor, the lymphocyte count in RZh was smaller than after transplantation of the other strains.

Levels of SDH activity in energy metabolism, characterizing the state of oxidative processes in the Krebs' cycle, and of GPDH activity, reflecting the connection between glycolysis and respiration, changed after transplantation of all strains except Mel-1, in which GPDH activity had only a tendency to rise. In RL-1 and RL-4, RZh, RP, and RMP the level of oxidative activity (per lymphocyte) of GPDH was increased to 11.6-13.5 U compared with background

values. The increase in relative activity of SDH was most marked in FL-4 and RMP to 15 and 17.4 U, respectively, (background value 10 U), whereas after transplantation of the other strains there was only a tendency for it to increase.

However, levels of absolute activity of these enzymes fell by different degrees, because of the decrease in the lymphocyte count, depending on the depth of lymphocytopenia. The most significant decrease was observed in RHz (SDH from 62,500 to 20,000 U, GPDH from 48,700 to 20,300 U), in which the increase in relative enzyme activity could not compensate for the sharp decrease in absolute activity, due to the marked lymphocytopenia.

After injection of the preparation, the absolute SDH and GPDH activity fell considerably, corresponding to the depth of lymphocytopenia, after transplantation of all the strains, and the relative activity of the enzymes differed. Injection of the preparation caused virtually no change (relative to the control) in SDH activity in Mel-1, RL-1, RP, and RZh, but it fell considerably in RL-4 and, especially, in RMP, i.e., in tumors whose transplantation led to a marked increase in the relative activity of SDH compared with the background. As a result, in RL-4 it was at the level of activity observed in intact animals, whereas in RMP it was 45% lower.

Relative activity of GPDH, after injection of the preparation, was increased in MEL-1 and very slightly reduced in RL-4 and RMP, but after transplantation of all the strains it remained higher than in intact mice.

The small change in activity of the enzymes of energy metabolism after treatment may indicate that the preparation has a direct inhibitory action on the peripheral blood lymphocytes of nude mice. If it is considered that the functional state of lymphocytes reflects reactivity of the animal to the procedure, it is possible to explain the differences in the values of the peripheral blood parameters after transplantation of the different strains and to establish correlation between the sensitivity of the corresponding heterografts of human tumors to the preparation and lymphocytes of the carrier mice.

For instance, the marked decrease after treatment in the relative activity of GPDH and in particular, of SDH of the lymphocytes in RL-4 and RMP, which showed the greatest increase after transplantation of these tumors, may be evidence of marked inhibition of glycolysis and of the Krebs' cycle respectively, which is combined with the highest antitumor effect of the preparation on these models. Conversely, after transplantation of strain Mel-1, which was least sensitive to treatment, injection of the compound did not prevent the increase in activity of the glycolytic enzyme GPDH.

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