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EFFECT OF HIGH-ALTITUDE HYPOXIA ON EFFECTIVENESS OF TUMOR CHEMOTHERAPY

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It was shown previously that high-altitude hypoxia inhibits tumor growth [3, 6]. In the investigation described below the response of tumors to chemotherapy in a high mountain environment was studied in order to investigate the antitumor effect of combined action of a chemotherapeutic agent and high-altitude hypoxia. We were motivated mainly by the fact that cytostatic compounds depress the functions of the blood system and lower nonspecific resistance, whereas high-altitude hypoxia stimulates hematopoiesis and increases the reserve powers of the body [1, 8, 9].

EXPERIMENTAL METHOD

Experiments were carried out on 165 noninbred albino rats of both sexes weighing initially 100-120 g. A Guérin carcinoma (series I) or sarcoma 45 (series II) was inoculated subcutaneously into the right side of the animals in the city of Frunze (760 m above sea level). Eight days after inoculation half of the animals were taken up to the Tuya-Ashu Pass (3200 m above sea level). Both in the plains and in the mountains some animals were treated by chemotherapy and the rest were untreated. Rats with a Guérin carcinoma were treated by intramuscular injections of thio-tepa in a dose of 2 mg/kg every other day, starting on the 10th day after inoculation (seven injections). Rats with sarcoma 45, starting on the 12th day after inoculation, received daily intramuscular injections of 7 mg/kg cyclophosphamide for 2 weeks. The hemoglobin concentration, erythrocyte, leukocyte, and platelet counts, and the leukocyte formula were calculated by the usual methods. The carcinolytic activity of the blood serum [5] and the blood concentration of sulfhydryl (SH) groups [7] also were studied. In each series of experiments the rats were killed at the same times on Tuya-Ashu Pass and in Frunze: animals with Guérin carcinoma on the 23rd day, rats with sarcoma 45 on the 26th day after inoculation. At autopsy the body weight and the weight of the thymus and the tumor were determined. The percentage inhibition of tumor growth and index of effectiveness of chemotherapy [4] were calculated from the weight of the tumor.

EXPERIMENTAL RESULTS

Growth of the Guérin carcinoma in the control rats was inhibited by 53.3% by exposure for 14 days to high-altitude hypoxia. The weight of the tumor in the animals in Frunze was 22.74 ± 4.3 g, compared with 10.6 ± 2.78 g on Tuya-Ashu Pass ($P < 0.05$). In the high-mountain environment, treatment of Guérin carcinoma with thio-tepa was more effective. For instance, whereas in Frunze the weight of the tumor was reduced by 12.3 times under the influence of thio-tepa (1.85 ± 0.42 g), on Tuya-Ashu Pass it was reduced by 28.1 times (0.81 ±

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TABLE 1. Results of Treatment of Rats with Sarcoma 45 by Cyclophosphamide in the Foothills (I) and High in the Mountains (II) (M ± m)

Group of animals	Experimental conditions	Weight of tumor after treatment, g	% inhibition of tumor growth within groups	Relative weight of thymus, mg	Blood SH-groups, μmoles/liter
Treated rats	I	4,83±0,71		77±5,7	122,7±7,9
	II	2,26±0,29*	53,2	109±6,9*	175,8±10,8*
Control rats with tumor	I	11,05±1,3		114±9,9	158,8±7,9
	II	6,43±1,1*	41,8	176±11,1*	203,4±5,7*
Intact rats	I	—	—	192±14,2	188,0±10,8
	II	—	—	204±12,0	292,9±15,0*

*$P < 0.05$.

0.24 g); the percentage inhibition of tumor growth in this case was increased from 91.9 to 96.4 ($P < 0.05$). At the end of the experiment, at autopsy the weight of the rats treated with thio-tepa in the foothills was 95.6 ± 6.4% of its initial value, whereas in rats treated in a high-mountain environment it was 116.2 ± 4.8%. The character of the change in body weight of the animals indicates that the toxic action of thio-tepa was greater in the foothills than high in the mountains.

In the next series of experiments the effect of high-altitude hypoxia on growth of sarcoma 45 and on the results of its treatment with cyclophosphamide was studied. As Table 1 shows, in the high-mountain environment growth of sarcoma 45 was significantly inhibited both in control rats and in rats treated with cyclophosphamide. The index of effectiveness of cyclophosphamide treatment high in the mountains (4.89) was higher than in the foothills (2.29); the percentage inhibition of tumor growth under the influence of cyclophosphamide was correspondingly increased from 56.3 to 79.6. No significant difference was observed in the change in body weight of the animals during cyclophosphamide treatment: In the foothills it was 95.4 ± 4.5% of the initial value, high in the mountains 102 ± 4.3%.

Adaptation to high-mountain conditions prevented to a definite degree the involution of the thymus which was observed during growth of the transplantable tumor and chemotherapy. For instance, whereas on the 26th day after transplantation of the tumor the relative weight of the thymus in rats kept constantly in the foothills decreased by 40.6%, in rats carried up to the Tuya-Ashu Pass it decreased by only 8.2% (Table 1). Administration of cyclophosphamide in the foothills led to a reduction in weight of the thymus by 59.9%, but up in the mountains the decrease was 45.3%.

During growth of the tumor in rats kept in the foothills the blood concentration of SH-groups decreased by 15.5% and administration of cyclophosphamide intensified the decrease to 34.7% (Table 1). Keeping the animals on Tuya-Ashu Pass led to elevation of the blood SH-group concentration both in intact rats and in rats with tumors. The greatest increase in concentration of SH-groups during adaptation to the high-mountain environment was found in intact animals (by 35.8%). The level of SH-groups in the untreated rats taken up to Tuya-Ashu Pass was 203.4 ± 5.7 μmoles, 22.9% higher than in animals kept in Frunze. After the course of cyclophosphamide treatment high in the mountains the concentration of SH-groups in rats with tumors was 30.2% higher than in animals treated with cyclophosphamide in the foothills and did not differ significantly from the concentration of SH-groups in the intact control animals. The protective action of high-altitude hypoxia resulting from inactivation of blood SH-groups by cyclophosphamide was also accompanied by a decrease in toxicity of the compound on the composition of the blood cells.

The animals kept in Frunze, whether untreated or receiving chemotherapy, developed anemia during tumor growth: Their hemoglobin concentration was 88.9 ± 3.6 and 96.5 ± 2.0 g/liter respectively (compared with 121 ± 1.8 g/liter in intact rats); the erythrocyte count was $3.26 \times 10^{12} \pm 0.11 \times 10^{12}$ and $3.39 \times 10^{12} \pm 0.07 \times 10^{12}$ cells/liter respectively (compared with $4.32 \times 10^{12} \pm 0.05 \times 10^{12}$ cells/liter in the control). No decrease in the red blood cell parameters was observed in rats with tumors taken up to Tuya-Ashu Pass: The hemoglobin level in the untreated animals was 141 ± 2.2 g/liter compared with 135 ± 3.8 g/liter in animals treated with cyclophosphamide. The erythrocyte count in these animals was $4.43 \times 10^{12} \pm 0.06 \times 10^{12}$ and $4.24 \times 10^{12} \pm 0.1 \times 10^{12}$ cells/liter respectively. In the intact animals ex-

posed to high-altitude hypoxia the greatest increase was found in the hemoglobin concentration (160.2 ± 2.8 g/liter) and erythrocyte count ($4.93 \times 10^{12} \pm 0.08 \times 10^{12}$ cells/liter).

Growth of sarcoma 45 was accompanied by a decrease in the peripheral blood platelet count to $179.8 \times 10^9 \pm 10.7 \times 10^9$ compared with $277.4 \times 10^9 \pm 7.5 \times 10^9$ cells/liter in intact animals. Chemotherapy aggravated this decline to $153.4 \times 10^9 \pm 8.0 \times 10^9$ cells/liter. High-altitude hypoxia had a stimulating action on thrombocytopoiesis, as shown by an increase in the peripheral blood platelet count in animals of all groups. The platelet count in intact rats kept on Tuya-Ashu Pass was $371.0 \times 10^9 \pm 11.2 \times 10^9$, in the control animals with tumors it was $257.9 \times 10^9 \pm 9.9 \times 10^9$, and in rats receiving a course of cyclophosphamide it was $227.0 \times 10^9 \pm 16.3 \times 10^9$ cells/liter, i.e., 32% higher than in the same group in the foothills.

No significant difference was observed in the leukocyte count after a course of cyclophosphamide treatment in animals with tumors taken up to Tuya-Ashu Pass and in those kept constantly in Frunze. However, under high-altitude conditions the lymphocytopenia was less marked than in the foothills. In rats treated with cyclophosphamide high in the mountains the absolute lymphocyte count was $2.71 \times 10^9 \pm 0.18 \times 10^9$ compared with $2.13 \times 10^9 \pm 0.18 \times 10^9$ cells/liter in animals treated in the foothills ($P < 0.05$). The decrease in the absolute lymphocyte count in the foothills reached $0.54 \times 10^9 \pm 0.88 \times 10^9$ cells/liter, whereas high in the mountains their number did not fall below $1.12 \times 10^9 \pm 1.38 \times 10^9$ cells/liter. Under the influence of cyclophosphamide a decrease in the lymphocyte count by half or more — below 2.5×10^9 cells/liter ($5.14 \times 10^9 \pm 0.39 \times 10^9$ cells/liter in intact animals) was observed in the foothills in 68% of rats, compared with 35% high in the mountains ($P < 0.05$). In untreated animals with tumors kept permanently in Frunze, the absolute number of stab neutrophils ($0.23 \times 10^9 \pm 0.4 \times 10^9$) was higher than in rats carried into the mountains ($0.05 \times 10^9 \pm 0.2 \times 10^9$ cells/liter). This shift toward stab cells can be explained by the larger size of the tumors in rats kept in the foothills, and the severer toxic effects on account of necrosis and ulceration of the tumors. Keeping the animals high in the mountains led to an increase in the number of monocytes. Their absolute number in untreated animals with tumors on Tuya-Ashu Pass was $0.62 \times 10^9 \pm 0.075 \times 10^9$ compared with $0.31 \times 10^9 \pm 0.036 \times 10^9$ cells/liter in control animals in the foothills. After a course of cyclophosphamide treatment under high-mountain conditions the monocyte count was $0.36 \times 10^9 \pm 0.043 \times 10^9$ compared with $0.21 \times 10^9 \pm 0.04 \times 10^9$ cells/liter in the foothills. The increase in the number of monocytes indicates activation of the reticuloendothelial system under high-mountain conditions and its role in antitumor immunity [2]. Stimulation of the functional state of the connective-tissue system under high-mountain conditions also indicates an increase in the ability of the blood serum to destroy cells of the ascites strain of Ehrlich's adenocarcinoma *in vitro*. The carcinolytic index in intact animals in Frunze was 14.1 ± 1.2%, after a stay on Tuya-Ashu Pass for 20 days it increased to 24.6 ± 1.8%.

The results thus showed that high-altitude hypoxia in the mountains inhibits tumor growth, increases the carcinolytic activity of the blood serum, and enhances the effectiveness of antitumor treatment with thio-tepa and cyclophosphamide. Keeping animals high in the mountains modifies the toxicity of cyclophosphamide: The fall in the blood SH-group level does not take place, the development of anemia is prevented, and the thrombocytopenia and lymphocytopenia are less marked. The results indicate the increased effectiveness of chemotherapy of malignant tumor at high altitudes in the mountains.

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