ONCOLOGY

Experimental Study of the Effects of Low-Intensity Monochromatic Photodiode Radiation and Antitumor Efficiency of Cyclophosphamide Injected with Autoblood

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 151, No. 1, pp. 67-71, January, 2011
Original article submitted August 18, 2009

Experimental study of extracorporeal exposure of autoblood to red monochromatic photodiode radiation for improving antitumor efficiency of cyclophosphamide injected with autoblood was carried out on the model of sarcoma 45 cells ectopically transplanted to the lungs. Co-incubation of irradiated autoblood with cyclophosphamide and reinfusion of the blood to animals increased the antitumor efficiency of chemotherapy in animals with tumors.

Key Words: *red spectrum monochromatic photodiode radiation; cyclophosphamide; antitumor effect*

Due to the development of new technologies in photobiology it became possible to carry out extracorporeal irradiation of the blood. Photomodification of the blood was carried out mainly by UV or low-intensity laser [1-3]. On the other hand, it is known that low-energy red spectrum radiation is a promising biologically significant tool for correction of the regulatory systems in living organism stimulating natural mechanisms of tumor cell death [9]; in addition, it is a method stimulating the antitumor efficiency of cyclophosphamide (CP) under experimental conditions [10].

We studied antitumor activity of CP injected with autoblood extracorporeally pre-exposed to red noncoherent photodiode radiation (PDR) in animals with sarcoma 45 transplanted into the lungs.

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MATERIALS AND METHODS

Experiments were carried out on male rats (n=31; 250-300 g). All animals were transplanted sarcoma 45 cell suspension (0.5 ml; 2×10^6 tumor cells). The model of ectopic tumor growing in the lungs was reproduced in rats after Yu. S. Sidorenko *et al.* [8]. Tumor strain was a gift from Cancer Research Center of the Russian Academy of Medical Sciences (Moscow). Experiments started from week 3 after transplantation. The animals were divided into 4 groups. In groups 1, 2, and 3 (7 animals per group), the blood (0.3 cm^3) was collected from the subclavian vein into tubes and the same volume of glugicir was added. In group 1, the blood was exposed to PDR, incubated for 40 min at 37°C, and reinfused into the subclavian vein. In group 2, the blood was exposed to PDR, CP (40 mg/kg) was added after 15-20 min, the blood was incubated for 40 min at 37°C, and reinfused into the subclavian vein. In group 3, CP was added to intact blood, the blood was incu-

0007-4888/11/1511-0058

bated for 40 min at 37°C, and reinfused into the subclavian vein. Antitumor therapy was carried out twice at 7-day intervals. The PDR exposure was carried out extracorporeally using a red photodiode at λ=0.67 μ from the Spektr-LZ device in a dose of W=3.06 J/cm^2. Irradiation was carried out in a continuous mode. Group 4 animals with tumors (controls) received no treatment. The animals were decapitated during week 6 after tumor cell transplantation to the lung. Blood smears were prepared, cytochemical studies of cationic proteins by the lysosomal cationic test were carried out [4]. The mean cytochemical coefficient was evaluated by the method of Astolde and Werge [7] (in arb. units) in blood specimens stained with fast green azur A. At least 100 cells were counted in each specimen. The parameters of nonspecific adaptation reactions and intoxication indexes were evaluated [5,6]. Lung tissues specimens were fixed in 4% formaldehyde for 3 days, processed by routine histological methods, 3-μ sections were prepared and stained with hematoxylin and eosin, after which histological studies were carried out. The pattern of distribution of the studied signs was evaluated by plotting frequency polygon before statistical processing of the results. In our study, the distribution of the studied signs corresponded to the normal (Gaussian) and hence, the results were statistically processed using Student's *t* test ($p<0.05$).

RESULTS

The parameters of nonspecific adaptation reactions in animals of all groups are summed up in Table 1. In group 2, the thymus weight was 1.9 times higher than in the control, in the rest groups 2-fold higher. The thymus/adrenal weight coefficients (K), indirectly reflecting the balance of the two important subsystems (immune and endocrine), were minimum in group 3 (2.7 times lower than in the control) and maximum in group 2 (2.1 times higher than the control). In group 1, K did not differ from the control. The peripheral blood lymphocyte count was abnormally high in the control (lymphocytosis) against the background of total reduction of blood leukocyte count. Blood lymphocyte and total leukocyte counts in groups 1 and 2 were within the normal range. In group 3, these parameters were significantly reduced compared to the control and groups 1 and 2. Gastrointestinal hemorrhages were found in groups 3 and 4; punctate hemorrhages were also found in some group 1 animals, but they were less pronounced; no changes of this kind were found in group 2 animals.

The Calf-Kalif leukocytic intoxication index (LII) can be regarded as one of integral indicators of the immune status in a tumor carrier. The LII is the proportion of neutrophilic to the rest cell percentage [5]. Normal LII ranges from 1 to 3 arb. units [6]. In groups 1, 3, and 4 (control), LII was low (<1). In group 2, this parameter was close to the upper boundary of the normal range and was 15-fold higher than in group 1, 9-fold higher than in group 3, and 17-fold higher than in group 4. Hence, LII in groups 1, 3, and 4 confirmed the presence of inflammatory and destructive processes of various severity and suppression of antitumor immunity. High LII in group 2 attested to

TABLE 1. Parameters of Nonspecific Adaptation Reactions in Control and Experimental Animals (*M*±*m*)

Parameter	Group 1 (*n*=7)	Group 2 (*n*=7)	Group 3 (*n*=7)	Group 4 (*n*=10)
Tumors in the lungs	—	—	Small tumor nodules	Large tumor nodes
Thymus weight, mg per 100 g body weight	60.2±2.1[2,4]	147.1±3.4[1,3,4]	66.4±1.1[2,4]	76.8±2.3[1,2]
Adrenal weight, mg per 100 g body weight	14.6±2.7	14.9±2.3	38±1[1,2,4]	16.5±1.3[3]
Thymus/adrenal weight coefficient	4.1±2.4[2,3]	9.8±2.2[1,3,4]	1.7±0.2[1,2,4]	4.7±2.4[2,3]
Gastrointestinal hemorrhages	Partial	—	+	+
Peripheral blood lymphocytes, %	57.7±1.7[3,4]	62.8±4.7[3,4]	30.3±3.3[1,2,4]	98.4±1.3[1,2,3]
Peripheral blood leukocyte count/ml[3]	10 480±110[3,4]	10 266±207[3,4]	4386±146[1,2,4]	7336±74[1,2,3]
Calf-Kalif LII	0.21±0.40[2,3]	3.15±0.10[1,3,4]	0.34±0.02[2,4]	0.18±0.02[2,3]
Blood neutrophil CPr	1.74±0.10[3,4]	1.59±0.10[3,4]	0.70±0.03[1,2,4]	0.38±0.05[1,2,3]
Adaptation reaction pattern	Training	Activation	Stress	Stress

Note. $p<0.05$ *vs.* [1]group 1, [2]group 2, [3]group 3, [4]group 4.

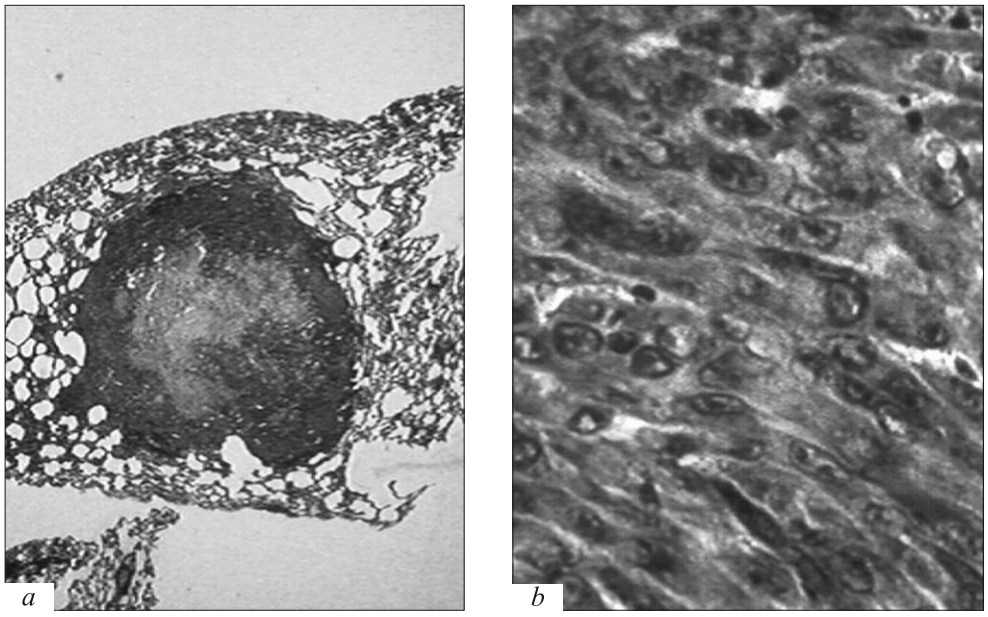

Fig. 1. Sarcoma 45, ectopically growing in the lungs of control rats. *a*) large tumor node with hemorrhages, ×10; *b*) typical spindle-shaped tumor cells with figures of pathological mitoses, ×40.

high functional potential of neutrophilic cells. These data are in good agreement with the results of lysosomal test (evaluation of cationic proteins, CPr, in blood granulocytes). CPr content was equally high in groups 1 and 2 surpassing 4-fold the control level and 2-fold the values in group 3. The increase of nonspecific antitumor resistance is paralleled by mobilization of the cellular component of the natural antitumor resistance, manifesting by an increase in the number and quality of neutrophils with a high level of CPr [8] and we therefore hypothesized that the level of nonspecific antitumor resistance could be modulated (increased) by PDR exposure, which really led to the formation of antistress nonspecific adaptation reactions. Study of the structure of adaptation reaction in each group showed high incidence of antistress reactions in groups 1 and 2: training and activation [2] (81 and 73%, respectively). Stress reaction predominated in groups without PDR exposure (98%).

Microscopic examination of histological preparations showed large foci of hemorrhages and numerous tumor nodes (0.5-1.0 cm) in specimens from the control group. Tumor invasion was found in the interalveolar septae, in the main respiratory structures, including the bronchioles. Tumor cells were presented by spindle, oval, and round cells with different figures of pathological mitoses. Connective tissue hyperplasia was seen in some places, forming a multinuclear layer of fibroblasts and histiocytes. Presumably, this process could be regarded as the construction of their own stroma by the tumor cells at the expense of the host lung material (Fig. 1). Foci of hemorrhages and signs of perifocal inflammation were found in the lungs of autopsied animals after PDR exposure and particularly after CP treatment; no large tumor nodes were detected. Microscopic analysis of lung tissue showed some very small nodules with modified tumor cells, loosing their spindle shape. Degenerative changes, manifesting by large vacuoles in the cytoplasm, hypochromism or disorderly formed chromatin reticulum in the nuclei

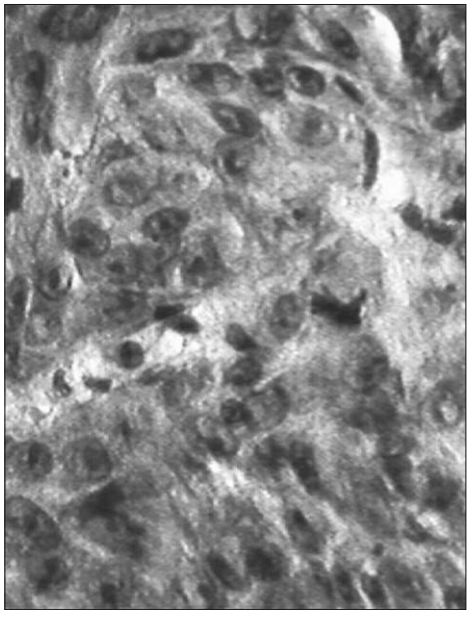

Fig. 2. Sarcoma 45, ectopically growing in the lungs of rats after intravenous injection of CP in autoblood. Small groups of tumor cells with degenerative changes, loosing their spindle shape, ×40.

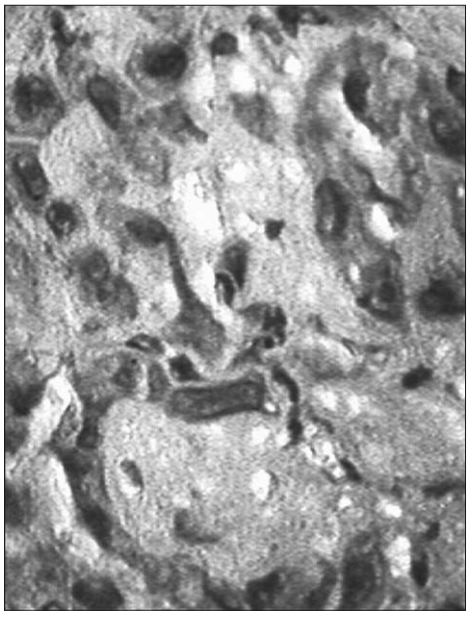

Fig. 3. Sarcoma 45, ectopically growing in the lungs of rats after intravenous injection of CP in autoblood pre-exposed to PDR. Scattered small groups and solitary round ghost-like tumor cells and polymorphic nude nuclei in sites of young connective tissue formation, ×40.

were found in tumor cells; karyorhexis, karyolysis, and other structural changes were found in the nuclei of some cells (Fig. 2).

The lungs of group 3 rats had no large or small nodular formations. Signs of interstitial and intraalveolar edema were found. Microscopic analysis of the lung parenchyma showed small scattered groups or solitary ghost-like tumor cells which lost their spindle shape. Numerous leukocytes and individual polymorphic nude nuclei were seen in sites of young connective tissue growth (Fig. 3).

These data indicate that destructive changes in the structure of tumors in the lungs (karyopyknosis, karyorhexis, lysis of tumor nuclei and cells, connective tissue infiltration, stimulation of the neutrophilic component and other immune system lymphocytic elements) result from at least two factors. First, local increase of antitumor resistance caused by the effector mechanisms of cell resistance, and second, resultant from common antistress nonspecific adaptation reactions formed at the whole-body level under the effect of PDR.

Hence, red light exposure in this mode stimulated the antitumor efficiency of CP injected in autoblood, promoting sarcoma 45 regression in the lungs and an increase of nonspecific antitumor resistance, which was shown by the highest values of cationic proteins and the integral criterion characterizing the total effect of experimental antitumor therapy.

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