Low-Energy Red Light Radiation Improves Antitumor Activity of Cyclophosphamide

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 138, No. 12, pp. 665-667, December, 2004
Original article submitted January 19, 2004

Exposure of rats with Pliss lymphosarcoma to incoherent red light according to certain protocols creates conditions preventing suppression of antitumor resistance, reducing the toxic effect of cyclophosphamide, and potentiating its antitumor effect.

Key Words: *red light; Pliss lymphosarcoma; antitumor effect*

Development of methods improving efficiency of antitumor drug and reducing their detrimental stress effects is an important problem of oncology. Low-energy red-band radiation is referred to prospective means for the correction of the status of regulatory systems [3,7]. Exposure to low-energy red laser according to some protocols produces a pronounced antistress effect [2,6] and improves antitumor efficiency of cyclophosphamide (CP) in experiments on c45 nonmetastasizing tumors [5,7].

Here we studied the possibility of improving antitumor activity of CP and alleviating its toxic effect by combining drug therapy with exposure to red light-emitting diode radiation (LEDR).

MATERIALS AND METHODS

The study was carried out on random-bred female rats (200±30 g). Group 1 consisted of intact animals (n=20). Other animals were transplanted Pliss lymphosarcoma (tumor cells were obtained from Cancer Research Center, Russian Academy of Medical Sciences). Tumor cell suspension (19×10^7) in 0.3 ml saline was subcutaneously injected on the back.

During tumor growth changes in tumor volume were recorded using Schreck formula for ellipsoid ($V=\pi/6\times abc$) with transformation by the formula $D=1.24X\sqrt[3]{V}$ in order to estimate the mean diameter of the tumor and evaluate the specific rate of its growth [8].

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Antitumor treatment was started on day 3 after transplantation. Cyclophosphamide was injected intraperitoneally in a dose of 50 mg/kg twice with 3-day interval. Red LEDR was emitted from a Spektr-LTs laser-light-emitting diode (LED) physiotherapeutic device for laser and selective LED photochromotherapy (PCT). The exposure was carried out using red LED at λ=0.67 μ. The maximum radiation power was 48 mW, maximum power density 7.5 mW/cm^2. The radiation was emitted in a continuous mode. The duration of one procedure was up to 3 min. A total of 8 procedures were carried out (daily in the same morning hours). Group 2 animals (n=20) were injected with CP, group 3 (n=20) received CP and LEDR exposure, and group 4 (n=20) received LEDR alone. Group 5 animals (n=20) with tumors served as controls.

Half of animals from each group were sacrificed on day 9; nonspecific adaptation reactions were tested [1,2], the absolute count of leukocytes per cm^3 was evaluated, tumor volumes were measured, and the antitumor effect was evaluated. Other animals were observed for 3 months after treatment. The dynamics of tumor growth, life span, presence of metastases by the moment of death were recorded.

The results were processed by methods of variation statistics using Student's t test.

RESULTS

Exposure to LEDR in the studied mode modified the tumor growth (Table 1). The initial volume of the tumor before the treatment (F0) was virtually the same

0007-4888/04/1386-0590

TABLE 1. Volume of Pliss Lymphosarcoma (cm^3) at Different Stages of Its Growth during PCT ($M\pm m$, n=10)

Growth stage	Day of observation	Groups			
		2	3	4	5
F0	Before experiment	0.34±0.10	0.31±0.20	0.32±0.10	0.31±0.20
F1	2	2.31±0.56	1.27±0.13[2]	2.65±0.66[3]	3.75±0.38[3]
F2	4	5.30±2.70	1.78±0.32[2]	8.64±0.87[2,3]	14.29±1.62[2,3,4]
F3	6	7.21±1.28	2.42±0.4[2]	18.17±1.12[2,3]	21.49±2.85[2,3]
F4	8	10.74±1.85	6.01±0.38[2]	24.44±0.95[2,3]	34.11±3.23[2,3,4]
F5	10	23.28±3.70	9.23±1.72[2]	40.18±1.43[2,3]	49.73±3.87[2,3,4]
F6	12	31.98±3.70	17.40±2.34[2]	58.25±2.11[2,3]	84.69±4.11[2,3,4]

Note. Here and in Tables 2 and 3: $p<0.05$ compared to the group shown by the upper index.

in the control and experimental groups. At stages F1-F4 tumor growth in the control surpassed the growth in all experimental groups (Table 1). At later stages (F5-F6) this trend was retained, the rate of tumor grown in experimental groups appreciably lagged behind the control, which characterized the dynamics of inhibition of Pliss lymphosarcoma growth.

By the end of observation the differences between tumor volumes, mean diameters, specific growth rate, presence of metastases, and life span were detected (Table 2).

The mean tumor diameter was maximum in the control. In groups 2 and 4 this parameter more than 2-fold surpassed that in group 3. Specific rate of lymphosarcoma growth decreased 2-fold *vs.* group 2 (CP alone) and 3.8 times in group 3 (LEDR). Metastases were detected only in the control and in group 2. The life span of control animals was 2-4.5 times shorter than in experimental groups; the maximum life span was observed in group 3 (Table 2).

Evaluation of nonspecific adaptation reactions showed that the thymus weight was significantly lower in the controls and in group 2 compared to other groups (Table 3). Coefficients of thymus to adrenals weight ratio (*C*) indirectly reflecting the balance between the immune and endocrine systems were minimum in groups 2 and 5 (4.5 times lower than in group 1). In animals with tumors (groups 3 and 4) *C* was 1.5 times lower than in intact animals, but 3-fold higher than in groups 2 and 5. Peripheral blood lymphocyte count was maximum in groups 3 and 4 and was comparable to that in group 1. Group 2 animals had the lowest values. Peripheral blood leukocyte counts in groups 3 and 4 did not differ significantly and were higher compared to groups 1 and 5 (1.4 and 1.8 times, respectively). Hemorrhages in the gastrointestinal tract were detected in animals of groups 2 and 5; in some animals in groups 3 and 4 hemorrhages were punctate and less pronounced.

These changes attest to profound disorders in the homeostatic processes during tumor growth and pronounced toxic effect of CP (group 2). Photochromotherapy normalized the adaptive mechanisms; antistress nonspecific adaptation reactions were observed.

A similar result was observed in our studies of the effects of low-intensity laser exposure and CP therapy

TABLE 2. Parameters of Pliss Lymphosarcoma Growth and Life Span during PCT ($M\pm m$, n=10)

Parameter	Groups			
	2	3	4	5
Mean diameter of tumor, cm				
before treatment	0.80±0.12	0.68±0.11	0.78±0.10	0.81±0.10
at the end of experiment	3.20±0.68	1.60±0.75[2]	3.80±0.76[3]	4.20±0.81[2,3]
Specific rate of tumor growth, days	0.38±0.20	0.18±0.10[2]	0.68±0.30[2,3]	0.81±0.20[2,3,4]
Tumor volume at the end of observation, cm^3	58.0±3.4	29.3±2.1[2]	98.6±2.1[2,3]	108.11±6.00[2,3,4]
Tumor growth, %	45	20	60	94
Growth inhibition, %	50	60	40	6
Tumor regression, %	5	20	0	0
Presence of metastases by death	Yes	No	No	Yes
Life span, days	40±4	96±7[2]	27.0±3.5[2,3]	21±3[2,3]

TABLE 3. Parameters of Nonspecific Adaptation Reactions ($M \pm m$, n=10-20)

Parameter	Groups				
	1	2	3	4	5
Thymus weight per 100 g body weight, mg	150.0±2.1	53.4±3.4[1]	139.8±17.4[1,2]	211.1±13.3[1,2,3]	87.8±3.1[1,3,4]
Adrenal weight per 100 g body weight, mg	22.4±2.7	36.7±2.3[1]	30.7±1.3[1]	46.3±1.7[1,2,3]	53.38±3.80[1,2,3]
Thymus to adrenal weight ratio	6.7	1.5[1]	4.6[1,2]	4.6[1,2]	1.6[1,3,4]
Hemorrhages in the stomach and intestine	No	Yes	Partial	Partial	Yes
Peripheral blood lymphocytes, %	65.0±0.8	31.3±3.3[1]	78.4±1.3[1,2]	62.8±4.7[2,3]	57.7±1.7[1,2,3,4]
Peripheral blood leukocyte count per ml[3]	11 883±102	6005±130[1]	16 350±150[1,2]	16 280±135[1,2]	8906±150[1,2,3,4]

in animals with c45 tumors [5]. No appreciable differences between the results of exposure to coherent and incoherent sources were observed at the organism level; that is, such characteristics as light coherence and polarization seem to be negligible in this case. Similar conclusions were made in a previous study [4].

Thus, PCT including red light exposure potentiated the antitumor effect of CP, reduced its damaging effects, and prevented metastases, stable leukopenia, and pronounced intoxication due to the formation of antistress adaptation reactions.

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