EXPERIMENTAL BIOLOGY

Effect of Monochromatic Light of Low Intensity on L929 Skin Fibroblast Culture

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 6, pp. 689-691, June, 2006
Original article submitted May 13, 2005

Effects of exposure to low-intensity monochromatic red spectrum radiation in different modes were studied on L929 skin fibroblast culture. Radiometric and cytological study of cell culture before and after irradiation showed that monochromatic low-intensity radiation stimulated skin fibroblasts in L929 culture under certain conditions.

Key Words: *red light; L929 skin fibroblast culture*

Similarly to lymphocytes, fibroblasts can migrate and contact with different cells forming a system of regulation and proliferation at the tissue level [4]. Experimental data on the effects of weak electromagnetic exposure of different spectrum bands on cell cultures were accumulated [6,8,9]. However, the effects of weak electromagnetic radiation of optical band on cell cultures, including skin fibroblast culture, remain unclear. The effects of low-intensity optic coherent (laser) electromagnetic exposure of tissues are studied best of all; the biological effects of this exposure on tissues with therapeutic purpose are particularly significant [5, 7]. The effect on reparative processes, including effects in combined treatment of tumors of different location, are evaluated depending on the type and dose of exposure. We evaluated the state of skin fibroblast and possible mechanisms of stimulation these cells with monochromatic low-intensity noncoherent optical irradiation on L929 culture of skin fibroblasts.

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MATERIALS AND METHODS

L929 mouse fibroblasts from Russian Specialized Collection of Cell Cultures, Institute of Cytology of Academy of Sciences of Russia (St. Petersburg), served as the object of optical exposure. L929 continuous cell line was pre-cultured in medium 199 with Eagle medium (1:1) with 10% FCS and 10 mg/ml gentamicin in Cartel flasks in accordance with requirements to manipulations with cell cultures [3]. Fibroblasts (1-1.5×10^5/ml) were inoculated in flasks with culture medium and coverslips on the bottom. After 24 h the coverslips with adherent fibroblasts were exposed to optical radiation, monochromatic noncoherent red light (λ=0.67 μ) generated by a Spektr-LC physiotherapeutic device (power density 7.5 mV/cm^2 and exposure duration up to 1 min). The study was carried out in 3 experimental and one control groups. Group 1 cultures were exposed to optical red radiation in a continuous mode, groups 2 and 3 cultures were exposed in a pulsed mode at pulse frequencies of 7.8 and 3.5 Hz, respectively. Estimated dose was within 0.75-0.9 J/cm^2.

Proliferative activity (PA) was studied and cytomorphometric analysis was carried out 48 h

0007-4888/06/1416-0738

TABLE 1. Effect of Optical Radiation of Low Intensity on L929 Cell PA (*M*±*m*)

Parameter	Control group	Experimental groups		
		1	2	3
Total cell count	2×10^4	2×10^4	2×10^4	2×10^4
Abs. number pulse/min	21 676.3±1139.0	51 350.9±5449.0^{*xo}	32 388.4±676.0^{*+o}	16 890±539^{*+x}
PA suppression or stimulation, %	—	-136.8	-49	22

Note. $p<0.05$ compared to *control, $^+$group 1, ^{x}group 2, ^{o}group 3. "—" PA stimulation.

after exposure in all experimental groups. PA was evaluated by ^{3}H-thymidine incorporation. Fibroblasts were placed into 96-well plates (2×10^4 cells/well in 0.1 ml). ^{3}H-Thymidine (185 GBq/mmol, 0.25 kBq in 0.05 ml) was added. After standard procedures the cells were transferred onto filters, treated with ethanol, thoroughly dried, and placed into vials with scintillation fluid (ZhS-8). Radioactivity (cpm) was counted for each filter using a β-scintillation counter. The mean for 4 repeats was estimated. The percentage of suppression or stimulation of ^{3}H-thymidine incorporation was evaluated by the formula: PA=(control cpm-experimental cpm) control cpm×100% [2]. Positive PA values indicated an inhibitory effect, negative ones a stimulatory effect of the studied factor. After the cell suspension was dried and stained after Romanowskii—Giemsa, cytomorphological studies were carried out on the preparations using a screw ocular micrometer and Avtandilov's ocular grid [1]. Cell counts per unit of standard area were estimated, caryometric measurements were carried out, and the volume of nuclei was estimated from these values using Arnoldi's formula [1]. Studies were carried out at 10×90 magnification; 10-20 tests were carried out in each case. The results were statistically processed using Student's *t* test.

RESULTS

Proliferative activity increased significantly in group 1 and to a lesser extent in group 2 (Table 1).

In group 3 PA values were positive, indicating suppression of the proliferative potential of cell culture after optic exposure in this mode.

Analysis of cytological preparations of L929 culture showed cells tightly adhering to the substrate; the cells were mainly round with basophilic cytoplasm, large (oval or bean-shaped) nucleus, with the nucleus/cytoplasm ratio of about 1. The greater part of morphometric parameters (cell shape, shape and volume of nucleus, nucleus/cytoplasm ratio) classify L929 cells as young fibroblasts.

After exposure to red light of low intensity fibroblasts transformed from round into spindle-shaped in all groups (Table 2), this process being most pronounced in group 2, which was seen from the ratio of cells with processes to round cells. Cell nuclei were well seen, their shape mainly ellipsoid, with even distribution of chromatin, well-

TABLE 2. Results of Morphometric Analysis of L929 Cell Culture after Exposure in Different Modes (*M*±*m*)

Parameter		Control group	Experimental groups		
			1	2	3
Total cell count per standard area		8.1±0.2	10.66±0.10^{*xo}	8.7±0.2^{*+}	8.84±0.2^{*+}
Elongated cells					
	abs. count	3.9±0.1	7.44±0.10^{*o}	7.2±0.1^{*o}	4.86±0.20^{*+x}
	%	43	69	83	55
Round cells					
	abs. count	4.2±0.3	3.22±0.50^{*x}	1.5±0.1^{*xo}	3.98±0.40^{x}
	%	52	31	17	45
Elongated/round cell ratio		0.92	2.23	4.88	1.22
Volume of nuclei, μ^3		1284.3±35.0^{+x}	1037±38^{*x}	828±35^{*+o}	1118.7±32^{x}

Note. $p<0.05$ compared to *control, $^+$group 1, ^{x}group 2, ^{o}group 3.

contoured nucleoli, and nucleus/cytoplasm ratio >1. In group 3 there were cells with hyperchromatic nuclei and low (below 1) nucleus/cytoplasm ratio; there were solitary fields of cells with nuclei lying alone. After exposure the parameters of the majority of cells corresponded to actively synthesizing fibroblasts. Their functional activity was also confirmed by detection of fine connective tissue fibers on the preparations in all experimental groups, but not in the control.

Hence, synthetic activity of *in vitro* cultured L929 cells increased and differentiation into mature collagen-producing forms was accelerated after exposure to red light in different modes. These data suggest that exposure to red light stimulates skin regeneration due to activation of fibroblasts. This aspect is important for the development of optimal protocols of optical exposure of the skin during treatment of various skin diseases.

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