EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Effect of Low-Intensity Laser Radiation on Peripheral Blood Eosinophils

O. G. Bondarenko and G. K. Popov

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The study was performed on peripheral blood eosinophils isolated on a Ficoll-Verografin continuous-density gradient. Low-intensity laser radiation (λ=0.89 μ, 25-30 mW power, 8 min exposure) induced degranulation of peripheral blood eosinophils. The effect of low-intensity laser radiation was realized via activation of calcium channels.

Key Words: *eosinophil; degranulation; laser radiation; verapamil*

Low-intensity laser radiation (LILR) produces a variety of therapeutic effects and is extensively used in medical practice. LILR in therapeutic doses causes modification of the surface structure of erythrocytes, platelets, and leukocytes in a small volume of blood leading to cell activation and modification of their properties and functions. The cells secrete cytokines and other bioactive substances and therefore the photomodified blood affects functional activity of various cells and involves practically all organism's systems in the reaction to irradiation.

Eosinophils regulate the vascular and infiltrative phase of inflammation. They modulate the release of histamine from basophils and mast cells and neutralize excessive amounts of secreted activated histamine. Eosinophils produce several enzymes involved in generation of antiinflammatory substances [2].

Here we studied the effect of LILR on functional activity of eosinophils.

MATERIALS AND METHODS

The study was performed on peripheral blood eosinophils (PBE). The cells were isolated from allergic patients with peripheral blood eosinophilia. Eosinophils from healthy donors served as the control. The peripheral venous blood (10 ml) was placed in a tube with

TABLE 1. Effect of LILR (0.89 μ) with Different Power for 1 and 8 min on PBE (*M*±*m*)

Duration and dose power of irradiation	Area, μ²	Specific optical density, arb. units
No treatment (intact cells)	883.9±44.1	16.3±0.7
1-min exposure		
7 mW	923.9±25.1	16.1±0.8
11 mW	887.5±39.6	16.6±1.3
17 mW	917.4±20.4	15.6±1.1
21 mW	893.3±17.3	17.1±0.4
25 mW	937.8±21.1	17.6±0.5
30 mW	919.4±37.6	17.2±1
8-min exposure		
7 mW	957.5±13.3	17.9±1.5
11 mW	897.7±4.9	16.2±1.1
17 mW	969.0±25.4	16.3±0.9
21 mW	988.8±17.1	16.6±0.8
25 mW	1053.3±22.9*	12.3±0.7*
30 mW	1119.9±18.4*	11.7±0.9*

Note. Here and in Table 2: *significant differences from intact cells.

Chelyabinsk State Institute of Laser Surgery. ***Address for correspondence:*** main@cgilh.chel.su. O. G. Bondarenko

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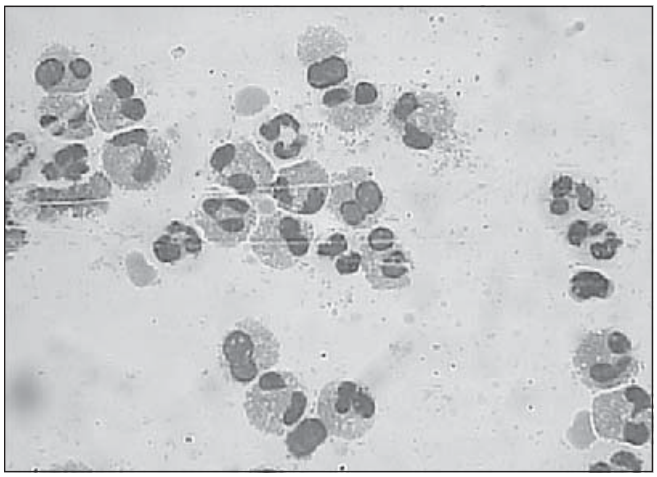

Fig. 1. Peripheral blood eosinophils before laser irradiation. Here and in Fig. 2: Romanovsky—Giemsa staining with azure and eosin, ×600.

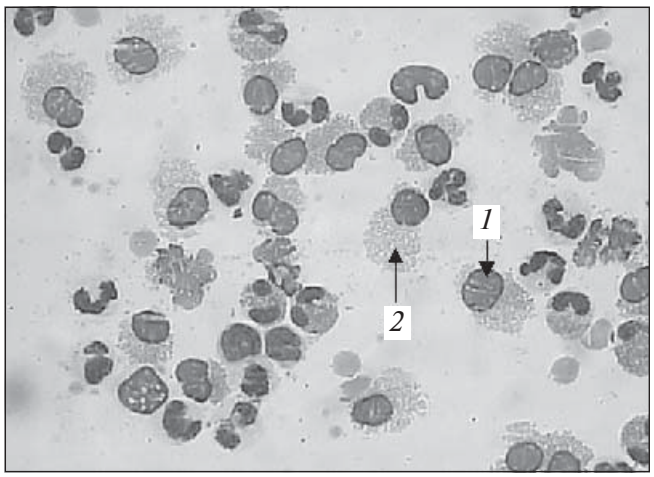

Fig. 2. Peripheral blood eosinophils after laser irradiation with 25 mW for 8 min. Changes in the shape and lightening of nuclei (*1*). Increase in the volume of cytoplasm (*2*) and cell.

heparin (500 U per 10 ml blood) and incubated at 37°C for 40 min. Eosinophils were isolated on a Ficoll-Verografin continuous-density gradient [1]. Ficoll-Verografin with a specific density of 1.120 g/ml (1 ml) was put in a siliconized centrifuge tube. Ficoll-Verografin solutions with decreasing densities of 1.115, 1.096, and 1.078 g/ml (1 ml each) were layered without mixing. The plasma was thoroughly layered onto the density gradient and centrifuged at 1500 rpm for 40 min. The cells from each density gradient were collected in individual tubes. Eosinophils (density gradient of 1.120 and 1.115 g/ml) were washed 3 times with 10 ml medium 199 at 1500 rpm for 5 min. The admixture of other blood cells was 20%. The suspension of eosinophils (10 μl) was mounted on a slide above a water bath (37°C) and irradiated. An Ulei 2KM laser was placed perpendicular to the slide at a distance of 5 mm. The wavelengths were 0.63 and 0.89 μ, the exposures 1 and 8 min, and the dose power from 7 to 30 mW. Smears were fixed and stained with azure and eosin by the method of Romanovsky—Giemsa. Smears of intact PBE served as the control. Viability of PBE was estimated by trypan blue staining [1]. The percentage of viable cells before and after LILR was more than 98%. Morphological characteristics of eosinophils remained unchanged after isolation.

Morphological changes were studied under a DMRXA microscope (Leika, ×1000) using DiaMorf Cito-W software. Morphometric study was performed to estimate the area and specific optical density of PBE.

The mechanism for LILR-produced degranulation of PBE was determined using verapamil in a concentration of 1.5×10^{-5} M. The suspension of PBE was irradiated 2 min after the addition of 2 μl verapamil.

RESULTS

LILR (λ=0.89 μ) for 1 min produced little effect on the area and specific optical density of PBE (Table 1).

Irradiation at 7, 11, 17, and 21 mW for 8 min also had no effect on PBE, while LILR at 25 and 30 mW for 8 min increased the area, but decreased specific optical density of PBE (Table 1).

TABLE 2. Effect of LILR (0.89 μ) on the Area and Specific Optical Density of Various Structures in PBE (*M*±*m*)

Duration and dose rate of irradiation	Area, μ²			Specific optical density, arb. units		
	nucleus	cytoplasm	cell	nucleus	cytoplasm	cell
Intact cells	191.9±2.7	281.0±3.7	479.6±3.6	30.9±0.4	9.8±0.4	18.1±0.5
1 min, 25 mW						
without verapamil	195.8±2.0	257.5±2.8	472.9±3.4	29.0±0.5	8.9±0.3	17.5±0.3
with verapamil	188.5±1.3	244.9±2.6	456.2±3.9	28.4±0.6	10.0±0.5	18.0±0.5
8 min, 25 mW						
without verapamil	255.0±2.2*	344.9±4.6*	599.4±7.3*	23.7±0.5*	6.5±0.3*	13.2±0.5*
with verapamil	201.1±2.1	235.3±3.5*	455.4±5.1	30.2±0.6	12.9±0.4*	19.8±0.5

PBE irradiated at 0.63 μ for 1 and 8 min did not differ from intact cells.

The effect of LILR on membrane structures of PBE was studied using calcium channel blocker verapamil. In this series we measured the area and optical density of the whole cell, and specific area and optical density of the nucleus and cytoplasm. These parameters insignificantly differed in verapamil-pretreated and intact PBE exposed to irradiation for 1 min. Significant differences were revealed between the area and specific optical density of the cell, nucleus, and cytoplasm in verapamil-pretreated and pharmacologically intact PBE irradiated at 0.89 μ for 8 min (25 mW, Table 2). Treatment of PBE with verapamil prevented degranulation of cells, which confirms published data that calcium channels play a role in LILR-produced exocytosis.

LILR at 0.89 μ for 8 min (25 mW) produced morphological changes in the shape of PBE nuclei (rounding and lightening, Figs. 1 and 2).

Our results show that LILR has little effect on degranulation of PBE at 0.63 μ, but stimulates exocytosis in cells at 0.89 μ. The degree of degranulation depended on the dose rate and exposure. Study with a calcium channel blocker verapamil indicates that the effect of LILR is mediated by activation of these channels.

REFERENCES

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