BIOPHYSICS AND BIOCHEMISTRY

Effect of Low-Intensity Laser Radiation on Leukocyte Function

G. I. Klebanov, Yu. O. Teselkin, I. V. Babenkova, T. Yu. Bashkueva, T. M. Modestova,* L. S. Steklova,* and Yu. A. Vladimirov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 4, pp. 395-398, April, 1997
Original article submitted January 22, 1996

The effect of a He-Ne laser (λ=632.8 nm) on the functional activity of peripheral blood leukocytes is examined by the method of luminol-dependent chemiluminescence. The maximum stimulation of leukocytes is observed in the 0.05-0.15 J/cm^2 range, being dependent on the leukocyte preparation. In the presence of the photosensitizing agent phthalocyanine the maximum stimulation is observed at lower intensities.

Key Words: *helium-neon laser; leukocyte priming; photosensitizing agent; luminol-dependent chemiluminescence of leukocytes*

Low-intensity laser radiation has found a wide application in clinical practice. Depending on the spectrum, dose, power, and other parameters, laser radiation has been used in antitumor phototherapy (damaging effect) [10,13] and therapy of some inflammatory diseases [4].

The damaging effect of laser radiation is based on the ability of exogenous photosensitizing agents (porphyrins, phthalocyanines, purpurins, chlorines, etc.) to induce free-radical reactions leading to cell death [14].

The mechanisms of laser stimulation remain unclear [1,2,5,8]. A positive effect of laser radiation (LR) in the absence of exogenous photosensitizing agents (PA) has been demonstrated in experiments on isolated cells [1,5,8]. For example, irradiation of leukocyte suspension with a He-Ne laser increased the production of active oxygen forms, cytokines, and ATP, stimulated cell receptors, and activated synthetic processes [5,7,9]. It should be noted that stimulating effects of LR are observed within a narrow dose range, outside which they disappear or are replaced by inhibitory, sometimes lethal, effects [5].

From these findings it can be suggested that both damaging and stimulating effects of LR are based on the same mechanism involving free-radical photosensitizing reactions, being determined by the content of endogenous PA which act as acceptors of laser energy.

The aim of the present study was to examine the effect of LR on functional activity of human peripheral blood leukocytes in the absence and presence of exogenous PA.

MATERIALS AND METHODS

Leukocytes were isolated [3] from 8-10 ml of peripheral blood of patients with bronchopulmonary disorders. Blood was obtained from the cubital vein after an overnight fast into a siliconized vial with heparin (20 U/ml). Cell suspension was stored in the cold for no more than 4 h.

Cells (10^5/ml Hanks' solution) were transferred into siliconized cuvette (diameter 1.8 cm) and ir-

Department of Biophysics, Russian State Medical University, *Alikor Center for Laser Therapy, Moscow

0007-4888/97/0004-0341$18.00 ©1997 Plenum Publishing Corporation

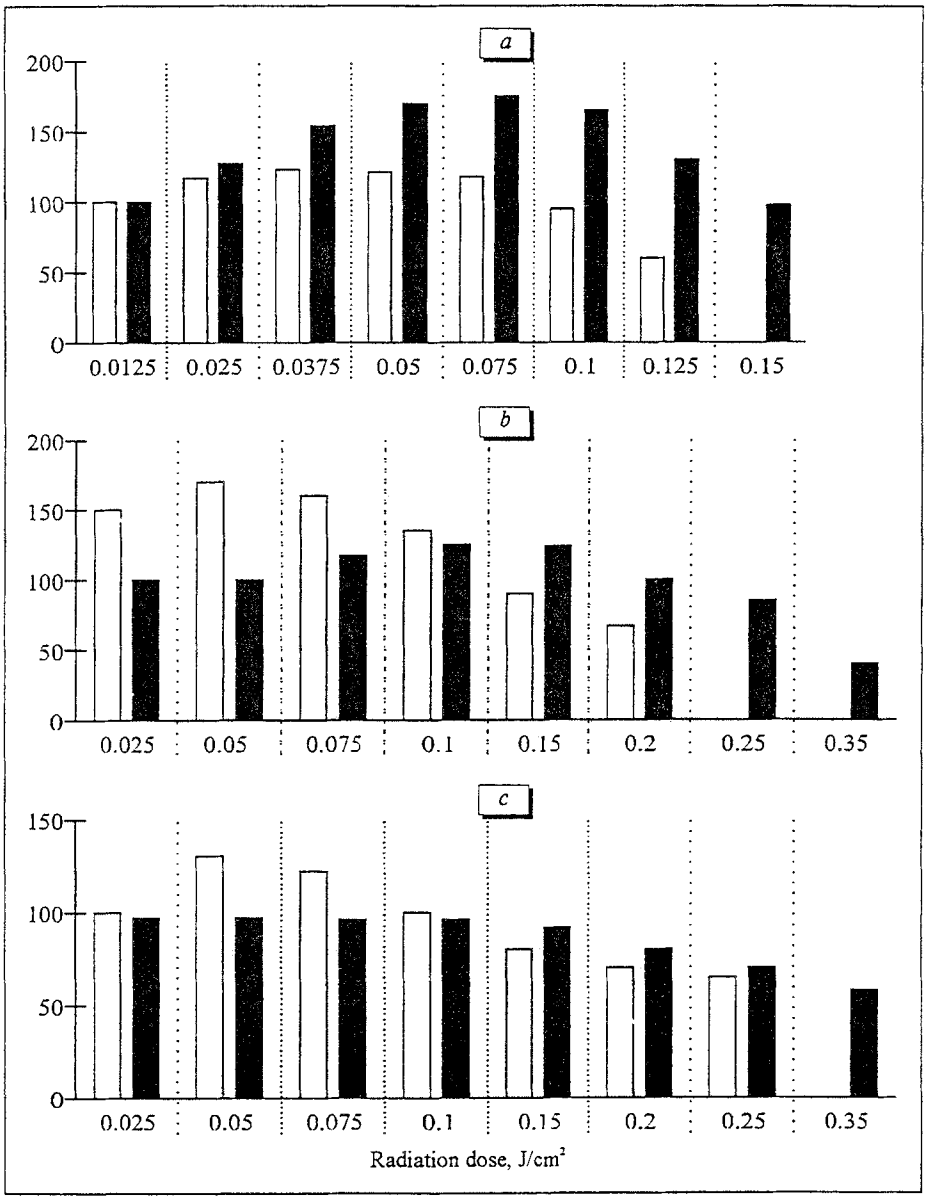

Fig. 1. Effect of laser radiation on the intensity of luminol-dependent chemiluminescence of peripheral blood leukocytes in the absence (dark bars) and presence (light bars) of the photosensitizing agent phthalocyanine. *a*) patients with acute pneumonia (n=4); *b*) patients with polysegmental pneumonia (n=2); *c*) patients with bronchitis (n=4). Ordinate: intensity of chemiluminescence, % of the control.

radiated from above with an ALTM-1 He-Ne laser (Dal'yus, Russia) at 632.8 nm, power at the cuvette bottom 0.17 mW, and light spot diameter 0.5 cm. The calculated radiation dose ranged from 0.025-0.5 J/cm^2. Irradiation was carried out at 37°C in a light-tight chamber with constant stirring. Phthalocyanine (Institute of Organic Pigments and Dyes, Moscow) was employed as an endogenous PA in a final concentration of 8.65×10^{-9} M. Control samples were incubated in the dark under the same conditions without laser irradiation.

Functional activity of leukocytes was assessed by measuring the intensity of luminol-dependent chemiluminescence (LCL) [3] in a KhLMTs-1 luminometer (Bikap, Russia). Luminol (Sigma) was added to a final concentration of 2.5×10^{-5} M. Measurements were carried out in Hanks' solution (4 ml). Spontaneous LCL was recorded for 2-3 min, after which opsonized zymosan (Sigma, 0.2 mg/sample) was added, and induced LCL was measured at 37°C with constant stirring. Functional activity of phagocytes (CL response) was expressed in relative units reflecting the difference between the maximum levels of stimulated and spontaneous LCL.

RESULTS

The CL-response of irradiated leukocytes varies depending on the severity of bronchopulmonary pathology.

In experiments with leukocytes from patients with acute pneumonia, the maximum LCL (77±13%

over the control) was observed at 0.05 J/cm² (1-min exposure, Fig. 1, *a*). Further increase in LR dose starting from 0.15 J/cm² resulted in a dose-dependent inhibition of the LCL response. When leukocytes from patients with acute phase of severe polysegmental pneumonia were irradiated, the maximum LCL (20±5%) was recorded at a higher dose (0.15 J/cm²) and longer exposure (3 min). Further increase in the dose led to inhibition of LCL (Fig. 1, *b*). Laser radiation had no appreciable effect on the CL response of leukocytes of patients with chronic bronchitis (Fig. 1, *c*).

These results suggest that after absorption by endogenous chromophores LR increases functional potential of phagocytes (Fig. 1, *a*, *b*). Endogenous porphyrins, whose content is changed in pathological states, may act as endogenous chromophores [8]. Porphyrins absorb a wide range of visible light [10, 13] and produce $^1O_2$ and other active oxygen species [8] that induce lipid peroxidation in the cell membrane, thus stimulating functional activity of leukocytes [11].

This hypothesis was tested in experiments with the exogenous PA phthalocyanine, which is employed in antitumor phototherapy [10,13]. Laser irradiation of leukocytes from patients with acute pneumonia in the presence of phthalocyanine reduced the CL response compared with the response of the same cells in the absence of this PA (Fig. 1, *a*). The maximum LCL in the presence of PA was observed at a lower LR dose (0.025 J/cm² *vs.* 0.05 J/cm² in the absence of PA). When the PA was added to cells with a low response to LR, the LCL response increased by 70±5%. It should be noted that the addition of PA increased the LCL response of leukocytes which did not react to LR (Fig. 1, *c*).

The LR-induced activation of leukocytes manifested itself only after stimulation with zymosan, which allows one to regard the effects of LR on leukocytes as priming [11]. An increase in the intracellular calcium content is the key stage of priming. Previously, we showed that incubation of peripheral blood polymorphonuclear leukocytes with the calcium ionophore ionomycin (10^{-9} M) caused their priming and increased the CL-response to opsonized zymosan [11].

It can be suggested that upon LR endogenous or exogenous PA induce lipid peroxidation in cell membranes, which leads to an increase in the intracellular calcium content with subsequent priming of leukocytes. Thus, LR modifies functional activity of phagocytes depending on their content of PA.

There is considerable clinical and experimental evidence indicating that dilation of microvessels and improvement of microcirculation are the major physiological effects of laser therapy [12]. Therefore, the ability of leukocytes and other cells to produce nitric oxide (NO) is particularly interesting [6]. It was reported that NO is a precursor of endothelium-derived relaxation factor (EDRF) [6]. The level of NO production is determined by functional potential of the cell, which is modified by priming.

Thus, the stimulating effect of laser radiation on leukocytes can be regarded as a result of the following events:

1. In some pathological states endogenous PA are inserted in the membranes of peripheral blood cells.

2. After absorption by PA, laser radiation causes priming of leukocytes with subsequent production of pro-oxidants and NO.

3. Nitric oxide forms EDRF that improves microcirculation, thus producing a beneficial effect on the organism.

An excess of endogenous PA results in inhibition of phagocytes or overproduction of NO, which may have dramatic consequences in certain diseases.

REFERENCES

1. A. M. Borisova, N. V. Khoroshilova, and T. I. Bulgakova, *Ter. Arkh.*, **64**, No. 5, 111-116 (1992).
2. Yu. A. Vladimirov, in: *Efferent Medicine* [in Russian], Moscow (1994), pp. 51-66.
3. Yu. A. Vladimirov and M. P. Sherstnev, *Itogi Nauki in Tekhniki, Ser. Biofizika* [in Russian], Vol. 24, Moscow (1989), pp. 30-44.
4. V. E. Illarionova, *Basic Laser Therapy* [in Russian], Moscow (1992).
5. T. Y. Karu, *Itogi Nauki i Tekhniki, Ser. Osnovy Lazernoi i Puchkovoi Tekhnologii* [in Russian], Vol. 4, Moscow (1989), pp. 44-84.
6. S. Kh. Snaider and D. S. Bredt, *V Mire Nauki*, No. 7, 16-26 (1992).
7. V. N. Shabalin, T. V. Ivanenko, T. V. Skokova, and N. Ya. Ol'shanskii, *Immunologiya*, No. 6, 30-32 (1990).
8. H. Friedmann, R. Lubart, I. Laulicht, and S. Rochkind, *J. Photochem. Photobiol.*, **11**, No. 1, 87-95 (1991).
9. J. O. Funk, A. Kruse, and H. Kirchner, *Ibid.*, **16**, No. 3-4, 347-355 (1992).
10. R. van Hillegersberg, W. J. Kort, and J. P. H. Wilson, *Drugs*, **48**, No. 4, 510-524 (1994).
11. L. V. Kovalchuk, G. I. Klebanov, S. R. Ribarov, *et al.*, *Biomed. Sci.*, **2**, No. 3, 11-22 (1991).
12. V. I. Kozlov, O. A. Termann, K. T. Zaitzev, *et al.*, *J. Clin. Laser Med. Surg.*, **10**, No. 2, 95-103 (1992).
13. H. I. Pass, *J. Natl. Cancer Inst.*, **85**, No. 6, 443-456 (1993).
14. J. T. Thomas and A. W. Girotti, *Biochim. Biophys. Acta*, **962**, No. 3, 297-302 (1988).