Mechanism of Therapeutic Effect of Low-Intensity Infrared Laser Radiation

G. I. Klebanov, M. V. Kreinina, E. A. Poltanov, T. V. Khristoforova*, and Yu. A. Vladimirov

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The effect of infrared low-intensity laser irradiation on functional activity of blood polymorphonuclear leukocytes was studied *in vitro*. A dose-dependent priming of polymorphonuclear leukocytes induced by infrared low-intensity laser irradiation was demonstrated. Similar effects were also observed in the presence of the photosensitizer photosense.

Key Words: *infrared laser irradiation*; *leukocytes; priming; luminol-dependent chemiluminescence*

Laser therapy (LT) of various inflammatory diseases uses mainly red and infrared light [6,13]. The positive effect of low-intensity laser irradiation (LILI) observed in laboratory and clinical practice is based on the following major physiological effects: stimulation of cell proliferation underlying molecular and cellular mechanisms of wound healing and treatment of arthrites and arthroses [1,14], improvement of blood microcirculation due to vasodilation, formation of new collaterals, and changes in rheological parameters of the blood [6,13].

Despite a large body of evidence on beneficial action of LT in clinical practice, little is known about molecular mechanisms of stimulating effects of LILI.

Previously we proposed the following concept of free radical mechanisms of stimulating effect of LILI (λ=632.8 nm) [3-5,12].

Endogenous porphyrins absorbing light in the red part of spectrum serve as the chromophores of laser irradiation. The porphyrins act as photosensitizers inducing free radical reactions (including lipid peroxidation with predominant formation of hydroperoxide of unsaturated fatty acids in phospholipids) after absorption of a quantum of light energy. Lipid peroxidation increases ionic permeability of plasma membranes (in particular, for calcium ions). The increase of intracellular calcium concentration in leukocytes activates Ca^{2+}-dependent processes and finally enhances functional potential of the cell. This so-called leukocyte priming can be detected by changes in luminol-dependent chemiluminescence (LDCL). Priming is accompanied by assembly of NADPH-oxidase components on the membrane in the waiting-active state and expression of surface receptors. Priming-induced enhancement of functional potential of leukocytes stimulates production of various bioactive agents such as $O_2^{\bullet-}$, H_2O_2, ClO^-, $NO^{\bullet}$, and various cytokines. Some of them possess bactericidal properties, while others affect blood microcirculation. All these factors improve blood flow in microvessels, which probably underlies most beneficial clinical effects of LT.

The above remarks relate to laser irradiation in the red band of the spectrum, where endogenous porphyrins could be the primary acceptors. However, there are reports on beneficial effects of infrared laser irradiation [6,13] with unknown primary acceptors. The hypotheses on participation of mitochondrial cytochrome-*c*-oxidase [10] or Ca^{2+}-ATPase [9] in absorption of infrared LILI does not explain the all clinical effects of infrared laser irradiation.

Our aim was to elucidate whether infrared laser irradiation modulates functional activity of blood leukocytes either in the absence or in the presence of photosense, an exogenous photosensitizer used in photodynamic antitumor therapy.

Department of Biophysics, Russian State Medical University, *Institute of Quantum Medicine, Moscow

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MATERIALS AND METHODS

Polymorphonuclear leukocytes from donor peripheral blood were used. Venous blood (10 ml) was stabilized with heparin (20 U/ml) and incubated at room temperature for 30 min. Leukocytes were isolated in a Ficoll-Verografin single-step density gradient [8]. Plasma with leukocytes was carefully layered on the gradient and centrifuged at 400*g* for 30 min. The upper fractions were carefully removed. The precipitate with polymorphonuclear leukocytes was resuspended in 10 ml phosphate buffer (pH 7.4) and centrifuged at 200*g* for 15 min. This washout procedure was repeated twice. The isolated leukocytes suspension were kept in Hanks' buffer (pH 7.4). The cells were counted in a Goryaev chamber. Leukocyte viability assessed by trypan blue exclusion test was not less than 97-98%.

Functional activity of leukocytes was assessed by luminol-dependent chemiluminescence (LDCL) [4,5, 12]. The LDCL kinetics was recorded on a KhLM-3 Chemiluminometer (Bikap, 3-ml cuvette) at constant stirring and constant temperature (37°C). First, luminol (Serva) was added to the cell suspension (10^6) in a final concentration of 3×10^{-5} M and spontaneous LDCL was measured. Then opsonized zymosan (Sigma) in a final concentration of 0.03 mg/ml was added to the sample and parameters of stimulated LDCL were recorded [4,5,12].

Treatment with LILI increases the maximum amplitude of LDCL (I) in comparison with the control (I_0) due to laser-induced leukocyte priming (Fig. 1). The effect of LILI on leukocytes was evaluated by priming index (PI) calculated by the formula: PI= $(I/I_0)\times100\%$.

An ALT Uzor infrared laser (Elektronika, λ=890 nm) was used. The experiments were carried out in a pulse-frequency mode (260 nsec pulse duration, 1 kHz modulation frequency, 0.3 mW mean beam power, and 0.4 mW/cm^2 radiation density). Immediately before LDCL recording, the leukocyte suspension was irradiated in chemiluminometer cuvette. The dose power varied from 0.012 to 0.120 J/cm^2. Controls samples before LDCL recording were incubated in a dark. In some experiments, the cells were incubated with photosense (exogenous photosensitizer, NIIOPIK, final concentration 10^{-9} M) in a dark or irradiated with the same doses, and after addition of opsonized zymosan LDCL was recorded. The control samples contained no photosense.

RESULTS

In experimental series I, the leukocyte suspension was subjected to LILI followed by stimulation with opsonized zymosan (Fig. 2, *1*). Increasing doses of laser irradiation enhanced LDCL of stimulated leukocytes. For instance, irradiation dose of 0.024 J/cm^2 increased PI by 2.5 times compared to the control. Higher irradiation dose produced a less pronounced effect: PI was about 120% for the dose of 0.1 J/cm^2. Similar biphasic changes of PI with increasing irradiation dose were previously observed after irradiation of leukocyte suspension with a He-Ne laser (λ=632.8 nm), [4,5,12]. In our previous studies photosense (agent used in photodynamic antitumor therapy) was applied for elucidation of the role of endogenous porphyrins in leukocyte priming [5,12]. Photosense produced opposite effects: in some experiments it increased PI of leukocytes at small doses of red LILI, while in others it decreased PI. These differences can be explained by the presence of small amounts of endogenous photosensitizing agent in leukocyte suspension in the first case, so the addition of photosense increased the total pool of photosensitizers to an optimal value and potentiated the effect of LILI on leukocyte priming. In the second case high concentration endogenous porphyrins in leukocyte suspensions resulted in hyperproduction of initiating radicals and decreased PI [5,12].

It was interesting to study the effect of photosense on irradiated leukocyte suspension. In these experi-

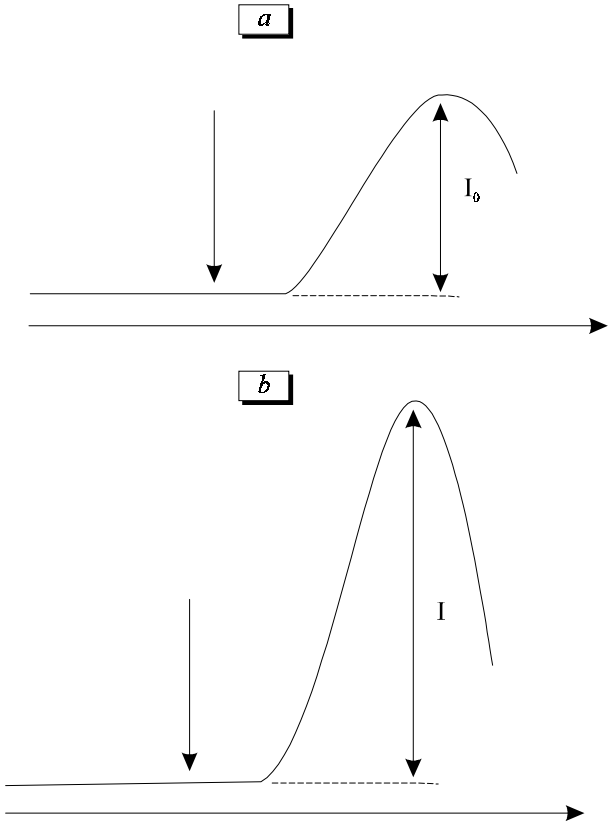

Fig. 1. A scheme of laser-induced leukocyte priming. *a*) control (dark incubation); *b*) experiment (preliminary infrared laser irradiation). Arrow shows addition of opsonized zymosan.

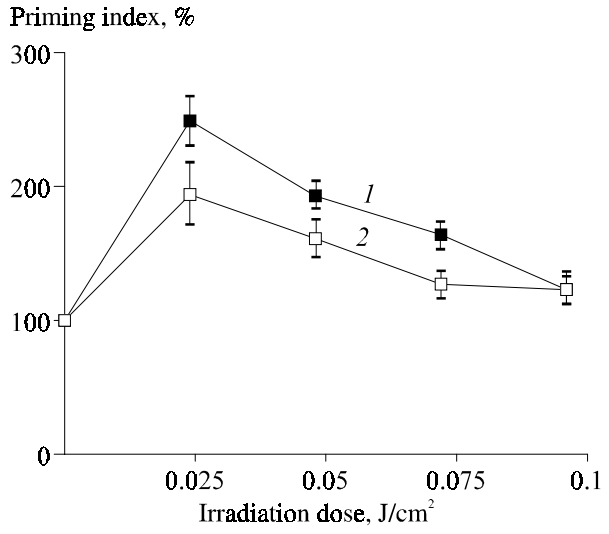

Fig. 2. Effect of infrared laser irradiation on functional activity of polymorphonuclear blood leukocytes in the absence (*1*) and presence of 10^{-9} M photosense (*2*).

ments the concentration of photosense was 10^{-9} M. Photosense in this concentration demonstrated no dark cytotoxicity.

Irradiation in the presence of photosensitizer induced leukocyte priming, and this effect was more pronounced at small radiation doses (Fig. 2, *2*). Further increase of LILI exposure inhibited functional activity of leukocytes. In the presence of photosense PI was lower for all irradiation doses. It can be assumed that blood leukocytes contained appreciable concentration of endogenous photosensitizers capable to absorb infrared laser radiation, so that their total concentrations surpassed a threshold content of chromophores needed for laser-induced leukocytes priming.

Our findings indicate that infrared laser irradiation induced leukocytes priming. Among many papers attesting to high therapeutic efficiency of LILI, there are only few experimental studies of the mechanisms of LILI action on cells *in vitro*. LILI was shown to increase proliferation index in fibroblasts [7]. Moreover, multichannel recording of absorption in cell monolayers revealed absorption bands at 670 and 820 nm [10]. Primary chromophore responsible for absorption of infrared light and initiation of secondary reactions can be mitochondrial cytochrome-*c*-oxidase [10]. The laser-induced changes of physicochemical properties of this enzyme during hypoxia reactivate mitochondrial respiratory chain, generate transmembrane potential, and accelerate ATP synthesis.

In the present experiments, the leukocytes subjected to infrared laser irradiation were mostly neutrophils containing few mitochondria [13]. In addition, short-term priming of granulocytes results from modification of NADPH-oxidase, so it can develop without mitochondria. Therefore, participation of mitochondrial cytochrome-*c*-oxidase in LILI-induced priming of leukocytes seems to be improbable.

Previous studies demonstrated that dihydrobiopterin and some metabolites of endogenous porphyrin can act as primary chromophores in the infrared band of the spectrum [2]. Further studies will be aimed at the search for possible chromophores in the infrared range and elucidation of their role in the stimulating effects of LILI.

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