Low-Power Laser Irradiation Induces Leukocyte Priming

G I Klebanov[1], Yu O Teselkin[1], I V Babenkova[1], T Yu Bashkujeva[1], T V Chichuk[2] and Yu A Vladimirov[1]

1 *Department of Biophysics of Russian State Medical University, Moscow Russia*
2 *State Research Centre of Laser Medicine Moscow Russia*

Abstract. Laser radiation (LR) of various spectral composition has been broadly used in clinical practice However the mechanism of the stimulating effects of LR remains obscure The effect of He-Ne LR (633 nm) on human blood leukocytes was investigated both in the absence and presence of 8 65 nmol/l phthalocyanine (PhC) Irradiation of non-stimulated leukocytes with 0 025 to 0 5 J/cm^2 did not lead to any activation of their luminol-dependent chemiluminescence (LCL) On the other hand LR increased in most cases the subsequent CL response of the cells to opsonized zymosan (priming action of He-Ne-laser light) The effect of LR on the leukocytes was not standard In irradiated leukocytes isolated from patients with severe acute or chronic pneumonia or chronic bronchitis the maximal LCL exceeded that for non-irradiated cells by 80% (0 05 J/cm^2) 20 25% (0 15 J/cm^2), and 0%, respectively (doses are shown in parentheses) Further increase of the exposure brought about a dose-dependent inhibition of LCL in cells from patients with severe acute and chronic pneumonia There was an intriguing relationship between maximal CL responses of leukocytes subjected to laser irradiation in the presence and without PhC When the priming effect of LR on isolated cells was small it increased in the presence of exogenous photosensitizer, phthalocyanine, in cells of severely ill patients where the initial effect of LR was strong Pc inhibited the priming action of LR Apparently, different cells contained different amounts of endogenous photosensitizer(s), the addition of exogenous sensitizer increased the priming action of LR at low concentrations and decreased it at higher concentrations of the endogenous photosensitizer

Key words: Laser — Polymorphnonuclear leukocytes — Priming — Photosensitization Phthalocyanine — Chemiluminescence Luminol

Correspondence to Professor G I Klebanov, Department of Biophysics of Russian State Medical University, Ostrovitjanova Str 1, 117869 Moscow, Russia E-mail gavlad@fbm msu ru

Introduction

Laser radiation of various spectral composition has been broadly used in clinical practice (Belkin and Schwartz 1989, Illarionov 1992, Wheeland 1993 Kozlova et al 1994) Depending on dose, power and other conditions radiation may produce damaging or beneficial effect on the organism (Sundberg et al 1981 Karu 1990 Hampton and Selman 1992, Yamamoto et al 1992) The destructive action of laser radiation (LR) used in photodynamic therapy (PDT) of tumors (Pass 1993) may be reasonably explained by the ability of exogenous sensitizers (porphyrins and phthalocyanines) to initiate free radical reactions in tumor cells that give rise to cell destruction (Van Steveninck et al 1986) In contrast, the mechanism of stimulating effects of LR is still a matter of hypotheses (Karu 1989 Zavarov et al 1989 Lubart et al 1990 Borisova et al 1992 Vladimirov 1994) The positive effect of LR in the absence of an exogenous sensitizer was revealed in studies on functional cell activity Increased production of reactive oxygen species (ROS) (Lubart et al 1990) cytokines (Funk et al 1992) and ATP (Romanova et al 1993) has been revealed upon irradiation with helium-neon laser *in vitro* along with receptor system expression (Shabalin et al 1990) and biosynthetic processes activation (Lubart et al 1992) The stimulating effects of LR can only be detected in a narrow range of incident doses, at higher doses the effects disappear and inhibitory effects are observed, which in many cases end up in cell death (Friedman et al 1991, Lubart et al 1992)

It may be proposed that both damaging and stimulating effects of laser radiation are produced via the same mechanism which involve photosensitized free radical reactions (Lubart et al 1990 1992 Friedman et al 1991), whereas the final effects may be opposite depending on the concentration of energy acceptors, i e endogenous sensitizers If sensitizers are abundant (as in case of the PDT), then excessive production of radicals formed gives rise to cell damage, while at low concentrations of (endogenous) sensitizers cell stimulation may occur

Among other targets of laser photons in the human blood, leukocytes may be of particular interest since they represent the principal antibacterial defense system in our body and are involved in local blood circulation control (Snader and Bredt 1992) It was the purpose of the present paper to study the modulation of the functional activity of human blood leukocytes upon laser irradiation in the presence and absence of exogenous photosensitizer

Materials and Methods

Isolation of leukocytes

Leukocytes were isolated from 8–10 ml of whole blood (Boyum 1968), taken from the cubital veins of patients suffering from broncheo-pulmonary pathology The

blood was taken in the morning on the empty stomach and placed into siliconized tubes containing heparin at 20 units/ml blood. After centrifugation for 10 min at 400 × *g*, the plasma was removed and 1 ml of 0.83% ammonium chloride was cautiously layered over the leukocyte button. The cell suspension was carefully collected with a syringe equipped with a long needle, and transferred into a tube containing 10 ml of 0.83% NH_4Cl, where the cells were incubated for 5 min at room temperature to hemolyse the contaminating erythrocytes, and subsequently sedimented by centrifugation at 400 × *g* for 10 min. All subsequent manipulations were performed using cooled solutions (4°C). The cells were suspended in 10 ml of a solution containing 2.7 mmol/l KCl, 136.7 mmol/l NaCl, 1.5 mmol/l KH_2PO_4, 8.1 mmol/l NaH_2PO_4, pH 7.4, and then centrifuged at 400 × *g* for 10 min. The washing procedure was repeated twice. The final pellet was resuspended in 1 ml of standard Hank's solution, and after cell counting in Gorjaev chamber it was diluted with Hank's solution to a final cell concentration of $2 \cdot 4 \cdot 10^6$ cells/ml. The neutrophil cells viability always exceeded 97% as determined by trypan blue exclusion. The cell suspension was kept cold and used within 6 hours.

Leukocyte irradiation with He-Ne-laser light

Laser dental instrument ALTM-1 (Dalvus, Moscow, Russia) was used as a source of radiation. The light beam (wavelength 632.8 nm) was directed on the object using fiber optics. Leukocytes were irradiated in a siliconized glass cuvette. The tip of the light-conductor was fixed above the leukocyte suspension, at a distance of 1.5 cm from the cuvette bottom. The volume of the irradiated sample was 1 ml, the leukocyte layer depth was 3.4 mm. The power of the laser radiation at this level measured with a RBK-7101 dosimeter (Anod, Moscow, Russia), was 0.17 mW, the light spot diameter was 0.5 cm. The doses, calculated from these data and the exposure times, varied from 0.025 to 0.5 J/cm^2. The irradiation was performed in a light-tight chamber at 37°C and under continuous agitation. The control sample was incubated under the same conditions but without laser illumination. In experiments with exogenous sensitizer, phthalocyanin (NIOPIC, Moscow, Russia) was additionally introduced into the sample in a final concentration of 8.65 nmol/l. For this purpose a certain amount of 0.2% solution of tetrasulfonate of aluminum phthalocyanine in 0.9% NaCl (Photosense, NIOPIC, Moscow, Russia) was added to the cell suspension. It has been shown in special experiments that in this concentration phthalocyanin did not influence the luminol-dependent chemiluminescence of leukocytes.

Measurement of luminol-dependent chemiluminescence

The functional activity of leukocytes was estimated by measuring the luminol-dependent chemiluminescence (Vladimirov and Sherstnev 1989) using a CLMC-1 luminometer (BIKAP, Moscow, Russia). All measurements of leukocytes LCL were

performed at 37°C under continuous agitation About 100 000 cells were placed into the chemiluminometer cuvette and the volume was adjusted to 4 0 ml with Hank s solution Then luminol (Sigma Deisenhofen, Germany) was added to a final concentration of 5 10 ^{5} mol/l and spontaneous chemiluminescence was measured during 2 3 min After that 0 1 ml of opsonized zymosan (2 mg/ml) was introduced into the cuvette and stimulated chemiluminescence was recorded For opsonization 10 mg zymosan was incubated for 10 min at 37°C with 3 ml blood serum and 7 ml Hank's solution The chemiluminescent response was measured as the difference between maximal intensities of stimulated and background chemiluminescence and presented in arbitrary units Each measurement of a given cell preparation was performed no less than 3 times and the mean value of the CL response was taken

Results

Leukocytes isolated from human blood, contain polymorphonuclear leukocytes (PMNL) or granulocytes lymphocytes and monocytes It has been shown earlier that the major part of the leukocyte chemiluminescence is due to PMNL which contribute 70% to the bulk cell amount in the leukomass (Vladimirov and Sheirstnev 1989)

A typical experiment is shown in Fig 1 Leukocytes were placed into the chemiluminometer cuvette and irradiated by He-Ne laser for 1 to 4 min under continuous agitation Then the laser beam was closed and the shutter of the chemiluminometer was opened Spontaneous chemiluminescence of the cells was monitored during

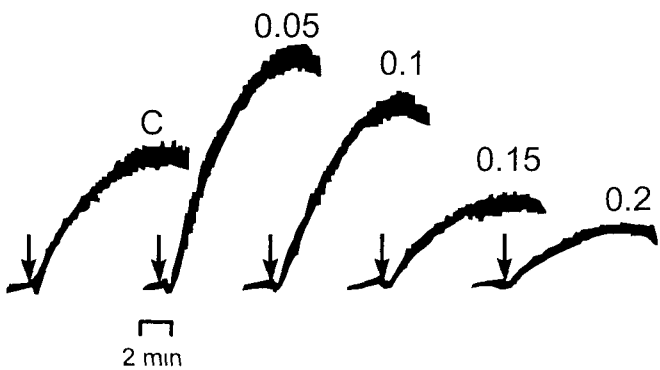

Figure 1 Typical changes in luminol dependent zymosan-stimulated chemiluminescence of leukocytes Leukocytes were isolated from the blood of patients with acute pneumonia and irradiated with He Ne-Laser light The doses of irradiation (J/cm^2) are indicated at the curves The chemiluminescence of irradiated leukocytes and of those incubated in the dark (control) was stimulated by opsonized zymosan (arrows) The chemiluminescence level before the zymosan introduction represents spontaneous (background) CL

1 2 mm Then, zymosan was introduced into the cuvette and the CL-response of the cells was recorded for subsequent 8–10 min In control experiments, leukocytes were incubated in the cuvette before zymosan addition in the dark over same period as in experiments with laser irradiation The spontaneous CL of the cells was in all cases rather low (several percents of the maximal CL amplitude after stimulation) and did not detectably differ between irradiated and non-irradiated samples In contrast, zymosan induced CL response was essentially different in non-irradiated and irradiated leukocytes For example, at the dose 0 05 J/cm^2, the amplitude was by 70% higher than in control, while at higher doses it decreased down to only 40% of the control at the dose 0 2 J/cm^2

Figure 2 shows the dose dependence of the effect of laser irradiation on the cell chemiluminescence response amplitude upon subsequent addition of opsonized zymosan for cells of patients suffering from different pulmonary diseases Control curves show the effects of laser irradiation on leukocyte suspensions in the absence of exogenous photosensitizers It is seen that the effect of laser radiation on the leukocyte functional activity was different in different blood samples In leukocytes isolated from patients with severe polysegmental pneumonia in acute phase (Fig 2A) the samples irradiated at the dose 0 05 J/cm^2 emitted chemiluminescence whose amplitude exceeded that of non irradiated cells (control) by approximately 80% Further increase of the exposure above 0 15 J/cm^2 brought about a dose-dependent inhibition of the CL response

In the second case, when leukocytes isolated from patients with chronic pneumonia were investigated (Fig 2B), the CL intensity of irradiated PMNL was higher

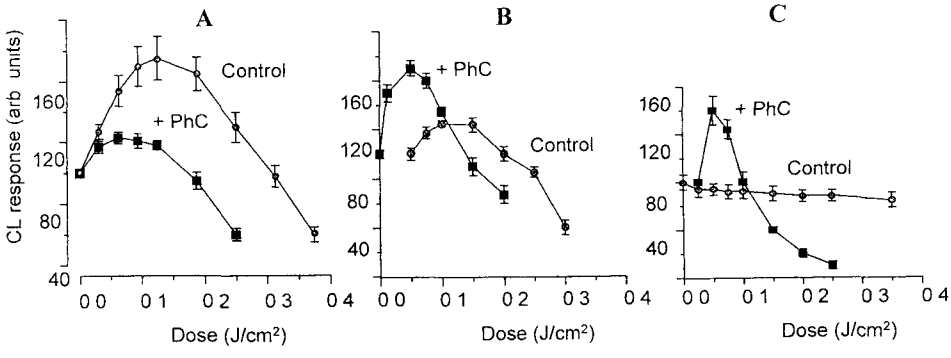

Figure 2. Effects of He-Ne-laser light on zymosan-induced CL response of leukocytes in the presence or absence of 8 64 × 10^{-9} mol/l phthalocyanine (PhC) Ordinate amplitude of zymosan-stimulated CL response (in %) to non-irradiated cells, abscissa irradiation dose (J/cm^2), A – patients with acute pneumonia (n = 7), B patients with chronic pneumonia (n = 5), C – patients with chronic bronchitis (n = 8) Each point represents the mean, error bar show standard deviation n – number of patients

only by 20–25% than in control, and was observed at higher doses (0.15 J/cm^2). Further increase of the dose again resulted in CL depression. It should be noted that at high doses of laser radiation, where the fall of CL response was observed, the percentages of died cells were negligible (3–5%, as measured with trypan blue test).

In the third case, when leukocytes isolated from patients with chronic bronchitis were subjected to laser irradiation, the latter did not produce any significant change in the CL curve shape or amplitude (Fig. 2C).

The observed enhancement of blood leukocyte functional activity appeared only as a result of the subsequent action of the stimulus. We did not ever observe a direct response of the cells to laser irradiation at the doses used. This means that laser radiation was a priming agent rather than a stimulus in all our experiments.

One explanation of the different effects of laser light on leukocytes can be differences in the concentrations of an endogenous laser energy acceptor, which we shall call *sensitizer* regardless of the mechanism of its action. If this is the case, addition of an exogenous sensitizer should produce priming effects, superimposed on those produced by the endogenous sensitizer.

To check this possibility we studied the effect of a well known photosensitizer, phthalocyanine, in combination with laser irradiation on the leukocyte CL response to the stimulus (opsonized zymosan). This dye sensitizes lipid photooxidation in liposomes (Klebanov et al. 1996) and erythrocyte ghosts (Zaidi et al. 1993) and is now widely used in photodynamic therapy of tumors (Pass 1993, Van Steveninck et al. 1986).

It has been found that laser irradiation of leukocytes in the presence of phthalocyanine enhanced the subsequent CL response of PMNL to stimulation with opsonized zymosan. At the same time, the maximum CL response was observed at lower doses of laser radiation (0.025 and 0.05 J/cm^2) than for cells which did not contain phthalocyanine (0.05 and 0.1 J/cm^2). Meshter et al. (1979) obtained similar results using methylene blue as photosensitizer.

The variations in the effects of laser irradiation on the leukocyte CL in the presence of Pc may be partially attributed to different binding of the dye to cells from different patient groups.

The inhibition of luminol enhanced chemiluminescence of PMNL in the presence of exogenous photosensitizer upon laser irradiation was also observed at lower radiation doses.

There is an interesting relationship between maximal CL responses of PMNL subjected to laser irradiation in the presence and without phthalocyanine. In cases when maximal CL response in the absence of sensitizer was weak, the effect of radiation was much higher in the presence of the dye (Figs. 2B and C). And *vice versa*, when the radiation effect was strong (Fig. 2A), the irradiation of leukocytes in the presence of phthalocyanine produced a very modest effect. In PMNL, in

which laser irradiation was ineffective in the absence of photosensitizer in the presence of phthalocyanine during laser irradiation was followed by a pronounced activation of the leukocytes

Discussion

Illumination of isolated blood leukocytes with He Ne laser light (632 8 nm) did not produce any activation of luminol-enhanced chemiluminescence in our experiments at any dose used (0 05 0 30 J/cm^2) On the other hand it was shown that laser irradiation of blood leukocyte suspension isolated from patients with pulmonary diseases in many cases essentially increased subsequent C L response of the cells to the stimulus (opsonized zymosan) In other words illumination of leukocytes with red (laser) light increased in certain cases and at a certain dose the potential of blood phagocytes to produce reactive oxygen species under subsequent stimulation A similar phenomenon produced by low concentrations of phagocyte stimuli has been described in the literature and called *priming* effect (Van Epps and Garcia 1980 Guthrie at al 1984) The essence of the phenomenon is that upon addition to phagocytes of very low concentrations of some compounds including FMLP FMA lipopolysaccharides, GM-CSF (Guthrie et al 1984 Woodman et al 1988) and calcium ionophores such as A23187 ionomycin (Finkel et al 1987 Kovalchuk et al 1991) to phagocytes, the respiratory burst (energetic reactive oxygen species production) is not observed but the cell response to the subsequent action of higher concentration of the same or another stimulus increases by a factor of 2 4 for neutrophils and up to 10 for macrophages (Woodman et al 1988 Kovalchuk et al 1991)

The crucial event in the priming effect is apparently an increase in intracellular calcium concentration The incubation of blood PMNL with the calcium ionophore ionomycin in concentrations of 2 4 10^{-9} mol/l brought about an enhancement of subsequent CL-response of the cells to the addition of opsonized zymosan (Kovalchuk et al 1991)

Also the incubation of neutrophils with lipid peroxidation products resulted in priming manifested in a 2 3 fold increase of subsequent CL-response at the same time the surface Fc-receptor concentration also increased by the same factor (Vladimirov et al 1990 Kovalchuk et al 1991) Lipid peroxidation was shown to increase ionic permeability of phospholipid and biological membranes including permeability for Ca^{2+} ions (Shubin et al 1975 Rubtsov et al 1984)

Hence we may hypothesize that in leukocytes containing a sensitizer, endo or exogenous, lipid peroxidation is initiated under laser illumination that, in its turn, increases cell membrane permeability to calcium ions and intracellular calcium concentration and brings about leukocyte priming On the other hand Zaidi et al (1993) have found that laser irradiation of microsomes and erythrocyte membranes

causes accumulation of lipid peroxidation products

The higher doses of laser radiation produced however, the opposite effect on the phagocytes In all cases, the priming effect was observed, if at all at comparatively low doses of laser radiation, while higher doses always depressed the cell activity (Karu 1990 Friedman et al 1991) It should be noted that increased intracellular calcium also usually produces cell activation at low and inhibits cell activity at higher Ca^{2+} concentrations (Sundberg et al 1981)

Whatever the mechanism of the priming action of laser light the principal problem is the nature of the primary photon acceptor the chromophore, that absorbs laser radiation and initiates photochemical reaction(s) Endogenous porphyrins may be proposed as such molecular acceptors of He-Ne-laser light (Chernitskiy and Slobozhanina 1989 Lubart et al 1992) porphyrin concentrations in tissues increase vary in some pathological states (Kuznetsova et al 1981, Grant et al 1993) porphyrins are known to absorb light in a broad range of the visible spectrum (Chernitskiy and Slobozhanina 1989) including 633 nm the wavelength of He-Ne-laser radiation

In the framework of this concept the variations of leukocyte CL responses upon laser irradiation may be accounted for by different contents of endogenous (and exogenous) sensitizers in the cells The higher the sensitizer concentration the more lipid peroxidation products are formed under irradiation producing first cell priming and then depression of respiratory burst upon stimulation When the concentrations of the endogenous photosensitizer is low or zero the addition of exogenous sensitizer causes a pronounced priming effect If the sensitizer concentration is initially high the exogenous sensitizer depresses cell activity rather than enhances it

The question of the clinical relevance of phagocyte priming upon laser irradiation is relevant in this context, since laser irradiation is applied in physiotherapy of a number of diseases including myocardial infarction, cardio-stenosis open wounds etc (Illarionov 1992, Kozlov and Buhin 1993) Clinical observations indicate that one important consequence of laser irradiation could be vasodilatation of small vessels (Snaider and Biedt 1992) followed by improved microcirculation (Kozlov et al 1990 Skobelkin 1990), and in particular giving rise to reperfusion facilitation after ischemia (Illarionov 1992) The ability of macrophages to produce nitric oxide along with reactive oxygen species (ROS) and other products is of particular interest in this respect Nitric oxide (NO) is a precursor of EDRF (endothelium-derived relaxing factor) (Snaider and Biedt 1992), and the level of its production should depend as in the case of ROS, on the cell activity which increases as a result of priming

In summary, the beneficial effect of laser therapy may be a result of the following chain of events (Fig 3)

1 The accumulation of an endogenous photosensitizer in the blood plasma

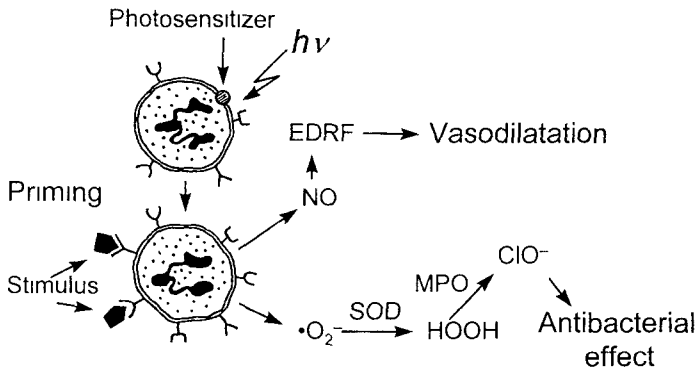

Figure 3. The consequences of the priming effects of laser radiation on blood phagocytes EDRF endothelium derived relaxing factor, NO nitric oxide, MPO myeloperoxidase, ClO^- hypochloric anion

and cells that depends on the organism state and a pathology development

2 Illumination of the cells with intense light in the spectral region of the sensitizer absorption induces priming in leukocytes that gives rise to increased production of ROS and NO by stimulated cells

3 EDRF, formed from NO, brings about vasodilatation effect and improves local and systemic blood circulation.

The excessive accumulation of the photosensitizer, as well as under the action of too high doses of laser light, the activity of phagocytes decreases and laser illumination produces negative rather than favorable effects Also, it cannot be excluded that excessive production of NO will also lead to exacerbation of the disease

Acknowledgements. This work was supported by Russian Foundation of Fundamental Research Grant No 97/04/4910

References

Belkin M , Schwartz M (1989) New biological phenomena assosiated with laser radiation Health Phys **56,** 687–696

Borisova D E , Horoshilova N V , Bulgakova G I (1992) Action of low-intensive laser irradiation on immune system Ter Archiv **64**, 111–116 (in Russian)

Boyin A (1968) Isolation of mononuclear cells and granulocytes from human blood Scand J Clin Invest **21** (Suppl 97), 77–89

Chernitskiy C D , Slobozhanina C I (1989) Spectral Luminescence Analysis in Medicine Nauka i Tekhnika, Minsk (in Russian)

Finkel T E Pabst M J Suzuki H Guthrie C A Forehand J R Phillips W A Johnston R B (1987) Priming of neutrophils and macrophages for enhanced release of superoxide anions by calcium ionophore ionomycin J Biol Chem **262** 12589 12596

Friedman H Lubart R Laulicht I Rochkind S (1991) A possible explanation of laser induced stimulation and damage of cell cultures J Photochem Photobiol **11** 87 95

Funk J O Kruse A Kishner H (1992) Cytokine production after helium neon laser irradiation in cultures of human peripheral blood mononuclear cells J Photochem Photobiol **16,** 347 355

Grant W E Hopper C MacRobert H J Speight P M Bown S G (1993) Photodynamic therapy of oral cancer photosensitization with systematic aminolevulinic acid Lancet **342** 147 198

Guthrie L A McPhail L C Henson P M Johnston R B Jr (1984) Priming of neutrophils for enhanced release of oxygen metabolites by bacterial lipopolysaccharide Evidence for increased activity of the superoxide producing enzyme J Exp Med **160,** 1656 1671

Hampton J A Selman S H (1992) Mechanisms of cell killing in photodynamic therapy using a novel *in vivo* drug/*in vitro* light culture system Photochem Photobiol **56** 235 243

Illarionov V E (1992) Basis of Laser Therapy Respect Moscow (in Russian)

Karu T I (1989) Photobiology of low power laser effects Health Phys **56** 691 704

Karu T I (1990) Effect of visible radiation on cultured cells Photochem Photobiol **52** 1089 1098

Klebanov G I Teselkin Yu O Babenkova I V Zhambalova B A Vandanmagsar B Nesterova O A Stranadko E F (1996) Effect of lipophilic antioxidants on peroxidation of liposome membranes photosensitized by hematoporphyrin derivates upon He Ne laser irradiation Membr Cell Biol **10** 139 143

Kovalchuk L V Klebanov G I, Ribarov S R Kremina M V Aptsiauri N E Gankovskaya L V Karaseva M V Shulkina E E Vladimirov Yu A (1991) Priming of phagocytes by cytokines and water soluble products of lipid peroxidation Biomed Sci **2** 221 231

Kozlov V I Bulin V P (1993) Basics of Laser Physiotherapy and Reflexotherapypp 54 55 Samara Kiev (in Russian)

Kozlov V I Terman O E Bulin V A Dementiyenko V R Krasyuk N Ya (1990) Changes in microcirculation in liver under low intensive laser effect In Laser Biophysics and New Application of Laser in Medicine (Ed O K Skobelkin) pp 139 141 Tartu University Tartu (in Russian)

Kozlova I S Tsirko V V Pirjazeva M A Volkova Z I Korjakina E V Nikolaeva V I Muldijarov P V (1994) The mechanism of laser therapy in rheumatoid arthritis Bull Exper Biol Med **66** 38 41 (in Russian)

Kuznetsova N P Panskov B S Chubarova A S Krivosheev B V Kapralov I K (1981) Porphyrins Medtsina Moscow (in Russian)

Lubart R Malik Z Rochkind S Fisher T (1990) A possible mechanism of low level laser-living cell interaction Laser Therapy **2,** 65 68

Lubart R Wolmann Y Friedman H Rochkind S Laulicht I (1992) Effects of visible and near infrared lasers on cell cultures J Photochem Photobiol **12** 305 310

Meshter C , Kozma L Dudash V Nitrai A , Deklen D (1979) Effect of laser irradiation on phagocyte activity of leukocytes Dokl AN BSSR **23**, 749 752 (in Russian)

Pass H (1993) Photodynamic therapy in oncology Mechanisms and clinical use J Natl Cancer Inst **85** 443 456

Romanova N D , Biovko L Yu , Sepulveda-Bessera E D , Ugarova N N (1993) Alteration of the pool of adenyl nucleotides in *E coli* bacteria cells under the action of low-intensive He-Ne laser Biokhimiya **58**, 376 384 (in Russian)

Rubtsov B V Klebanov G I Ruzhitskiy A O Vladimirov Yu A (1984) Comparison of the action of UV-radiation and Fe-induced lipid peroxidation on sarcoplasmic reticulum membranes Izv AN USSR Ser Biol **2**, 299–302 (in Russian)

Shabalin V N Ivanenko T V , Skokova T V Olshanskiy N Ya (1990) Immunological and physicochemical effects of laser action on biological objects Immunologiya **6** 30 32 (in Russian)

Shubin M V Poglazov A F , Skotselyas Yu G Vladimirov Yu A (1975) Studies on the effect of lipid peroxidation on liposome membrane permeability for Ca^{2+} ions Biofizika **20**, 161 163 (in Russian)

Skobelkin O K Kozlov V I , Litvin G D Bulin V P Azizov G A (1990) Blood microcirculation under laser- physio- and reflexotherapy in patients with lesion in vessels of low extremities Laser Therapy **2**, 69 78

Snaider S Biedt D S (1992) Biological role of nitrogen oxide V mire nauki **7**, 16 26 (in Russian)

Sundberg S Glette J Hopen G , Solberg C O Ramslo J (1981) Porphyrin-induced photodamage to isolated human neutrophils Photochem Photobiol **34** 471 475

Van Epps K , Garcia M (1980) Enhancement of neutrophil function as a result of prior exposure to chemotactic factors J Clin Invest **66** 167 175

Van Steveninck J Tijssen K Boegheim J P Vander Zee J Dubbelman T M (1986) Photodynamic generation of hydroxyl radicals by hematoporphyrin derivative and light Photochem Photobiol **44**, 711 716

Vladimirov Yu A (1994) Three hypotheses obout the mechanism of action of red (laser) light In Efferent Medicine (Ed S Ya Chikin) pp 23 35 Institute of Physico-Chemical Medicine Moscow (in Russian)

Vladimirov Yu A Sherstnev M P (1989) Chemiluminescence of Animal Cells Itogi Nauki i Tekhniki Ser Biofizika Vol 24 pp 74 100 Moscow (in Russian)

Vladimirov Yu A Ribarov S R , Bochev P G Benov L C , Klebanov G I (1990) Effect of oxidized phospholipids on the chemiluminescence of zymosan-stimulated leukocytes Gen Physiol Biophys **9**, 45 54

Wheeland R G (1993) Lasers for the stimulation or inhibition of wound healing J Dermatol Surg Oncol **19**, 747 752

Woodman R C , Curnutte J T , Babior B M (1988) Evidence that *de novo* synthesis participates in time-dependent augmentation of the chemotactic peptide-induced respiratory burst in neutrophils Effects of recombinant human colony stimulating factors and dihydrocytochalasin B Free Radical Biol Med **5** 355 361

Yamamoto N Homma S Nakagawa Y , Hayami M Imanaga H , Kurimoto M Mitsuhashi M , Kimoto T (1992) Activation of mouse macrophages by *in vivo* and *in vitro* treatment with cyanine dye, lumin J Photochem Photobiol **13**, 295 306

Zaidi S I , Agarwal R Eichler G Richter B D , Kennev M E , Mukhtar H (1993) Photodynamic effects of new silicon phthalocyanines *in vitro* studies utilizing rat

hepatic microsomes and human erythrocyte ghosts as model membrane sources. Photochem. Photobiol. **58**, 204–210.

Zavarov, S. D., Skopionov, S. A., Chudnovskii, V. M. (1989) Primary mechanisms of the action of low-intensive laser irradiation in biological systems. Weakly-absorbing acceptors and structural enhancement of local action in biological liquids. In: Lasers and Medicine (Ed. O. K. Skobelkin) pp. 81–82. Ministry of Health USSR, Moscow (in Russian).

Final version accepted October 30, 1998