Membranotropic Effect of Low-Intensity Laser Radiation of the Blood

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We have examined the effect of a low-intensity laser radiation (wavelength 630 nm, beam power 1 mW) on the aggregability of blood platelets, acidic tolerance of erythrocytes, and the structure of their membranes. Laser radiation suppressed platelet aggregability induced by ATP, epinephrine, collagen, platelet activating factor, and fibrinogen and enhanced the resistance of the erythrocytes. It decreased the microviscosity of lipid bilayer and of the membrane protein-lipid contact regions and slowed down lipid peroxidation. These findings attest to a direct membranotropic effect of laser radiation, which is particularly strong in the protein-lipid contact regions.

Key Words: *low-intensity laser radiation; membrane; blood platelets; erythrocytes*

Laser irradiation of peripheral blood has been widely used in medicine as an effective therapeutic method. Until recently many aspects of pathochemical and biophysical mechanisms of laser radiation remained unclear, which prevented unveiling its medical potential and dispersed doubts on its safety. The primary targets of intravascular irradiation are the blood cells. Many beneficial medical effects of laser radiation are probably caused by changes in the functional state of blood cells. The following events seem to occur upon laser irradiation of peripheral blood: stimulation of blood cells → anticoagulant [1,14] and fibrinolytic effects [V. I. Illarionov *et al.*, 1988] → amelioration of microcirculation [10] → anti-ischemic effect [11], detoxification, etc. [6]. The most obscure event is the interaction of laser radiation with biological molecules that leads to cell stimulation.

At least four mechanisms may be involved in the primary response to laser radiation: a nonspecific structural alteration of the biological fluid, photochemical activation of free-radical reactions (specifically, lipid peroxidation, LP); direct membranotropic effect with modification of lipid bilayer; conformational rearrangements in proteins with concomitant modulation of the receptor-mediated and metabolic reactions [12]. Our aim was to study the effect of low-intensity laser radiation (LILR) on the functional state of platelets, stability of erythrocytes, and structural properties of their membranes and membrane LP.

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MATERIALS AND METHODS

Blood obtained from 20 donors was irradiated *in vitro* and blood of 17 patients with nonspecific reactive hepatitis was irradiated intravenously. Blood was drawn from the cubital vein and stabilized with 3.8% sodium citrate. It was irradiated *in vitro* with constant stirring for 15 min with an ALOK-1 He-Ne laser (λ=630 nm, beam power 1 mW). Intravenous laser irradiation was performed with the help of a light guide inserted into the cubital vein [10]. The radiation course consisted of five 30-min sessions (one session every day). Blood was drawn after the radiation course. Platelet-rich plasma was prepared as described [13]. Platelet aggregability was studied according to modification [3] of method [16] using an 230LA dual-channel laser platelet

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aggregation analyzer (Biola, Russia). Platelet aggregation was induced with ADP (5 μM, Serva), epinephrine hydrochloride (5 μM), platelet activating factor (0.01 μM, Sigma), collagen (4 μg/ml, Sigma), ristocetin (1.2 μg/ml, Sigma), and fibrinogen (3 g/liter, Lithuania). Platelet aggregation was measured in percentage. The aggregation plots were processed with an AGGR-2.20 original computer software. Platelet morphology and count were determined with the help of a laser aggregation analyzer [3].

The structure of the erythrocyte membrane was studied by lateral diffusion of the fluorescent probe pyrene (Sigma). The samples were analyzed in a Hitachi 650-60 spectrofluorimeter at excitation wavelength 334 nm. The relative microviscosity of lipid bilayer was assessed according to the excimerization coefficient of pyrene which is equal to the ratio of intensity of fluorescence of excimers (F_E) and monomers (F_M) at the excitation wavelengths 334 and 286 nm [2].

The content of the substances that react with 2-thiobarbituric acid, was determined in plasma and membranes of erythrocytes by a modified method [5] in spectrophotometer (excitation wavelength 535 nm). Plasma ceruloplasmin content was determined as described [8]. The acidic tolerance of blood cells was measured by the method of I. A. Terskov *et al.*, 1967. The content of medium-sized molecules was evaluated according to extinction of protein-free extracts of blood plasma using an SF-46 spectrophotometer at λ=220 nm [4].

RESULTS

According to a modern biophysical concept, the therapeutic effect of laser radiation is not realized via any specific receptor mechanism and the biological fluids, but it involves a wide spectrum of effects on the biopolymers: proteins, lipoproteins, and structural components of biological membranes [9]. This variety may be responsible for the versatile antiaggregation effect of laser radiation, which resulted in approximately similar *in vitro* and *in vivo* inhibition of platelet aggregation induced by ADP, epinephrine, collagen, platelet activating factor, and fibrinogen (Fig. 1). The ristocetin-induced adhesion of the platelets was inhibited to a lesser extent, which is caused not only by platelet activation but also by direct interaction of their membrane structures [15].

It is noteworthy that spontaneous platelet aggregation was efficiently stabilized by laser radiation. The polyvalent decrease in platelet sensitivity to strong and weak inducers was not accompanied by changes in platelet count, although the platelet population was redistributed: the count of spherical (active) platelets decreased more than 3-fold, and there was a certain increase in the count of discoid (inactive) platelets. This attests to a stabilizing effect of LILR on the platelets.

Thus, LILR decreases the number of activated platelets in the blood and decreases their ability to be activated by various inducers. It is likely that LILR does not affect the receptors directly, while its antiaggregability effect may be associated with nonspecific membranotropic effect.

This hypothesis is consistent with the data on acidic hemolysis of erythrocytes. Irradiated erythrocytes were more tolerant to acid, which was manifested in slowing down of hemolysis (erythrogram being shifted to the right) and in a decrease of its intensity (Fig. 2).

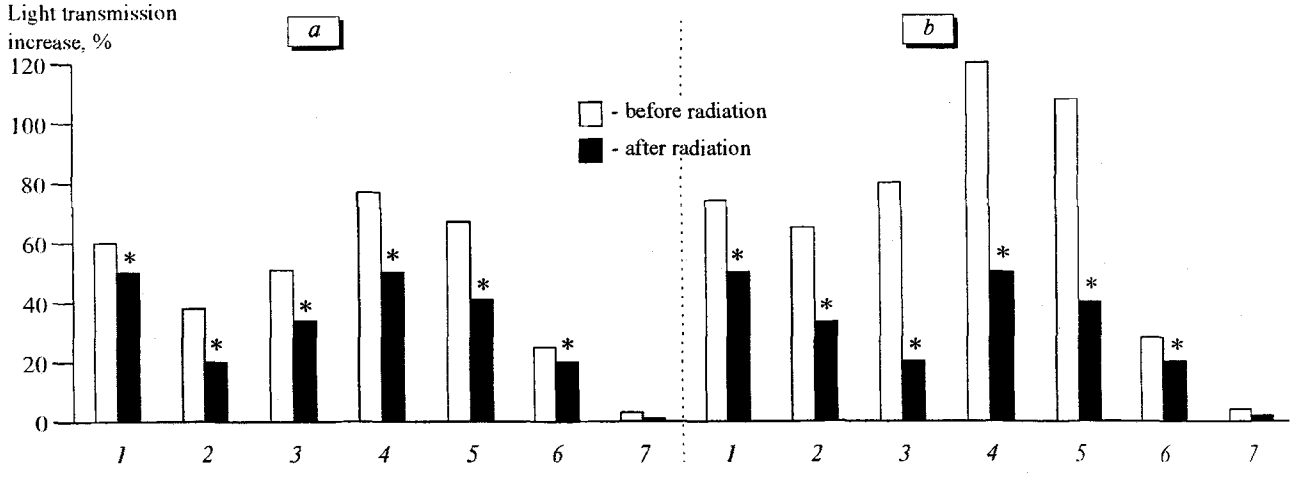

Fig. 1. Effect of low-intensity laser radiation of blood on platelet aggregation *in vivo* (*a*) and *in vitro* (*b*). Aggregation was induced by ADP (*1*), epinephrine (*2*), collagen (*3*), platelet activating factor (*4*), fibrinogen (*5*), and ristocetin (6). Number 7 corresponds to spontaneous aggregation. *p<0.05 relative to the data before radiation.

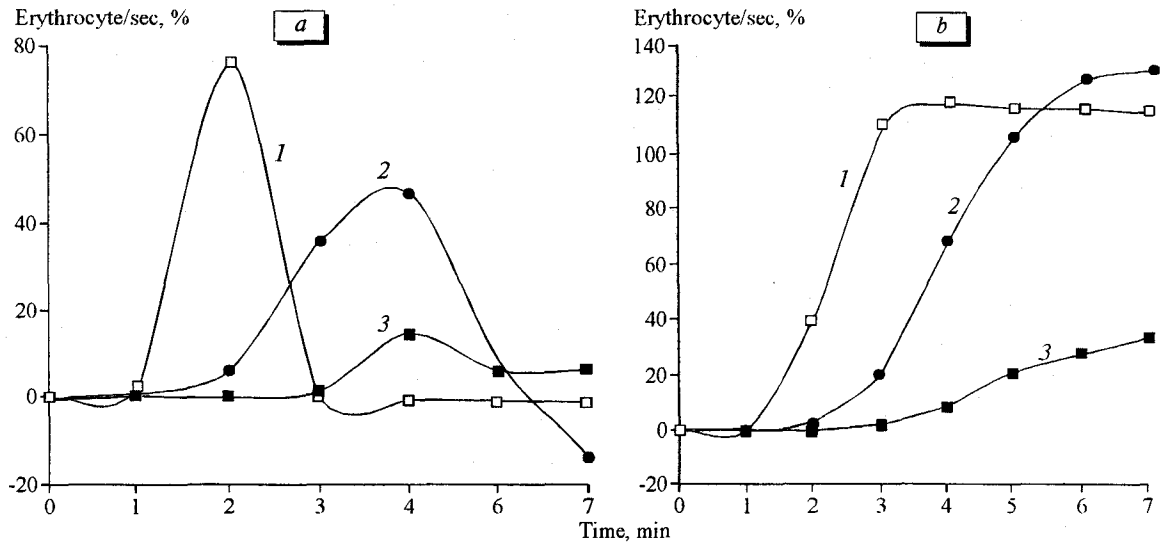

Fig. 2. Effect of low-intensity laser radiation of blood on acidic tolerance of erythrocytes before irradiation (*1*), 5 min (*2*), and 50 min (*3*) after irradiation. *a*) rate of hemolysis; *b*) degree of hemolysis.

The most probable interpretation of these data is based on the hypothesis that LILR induces rearrangement of the blood cell membrane. Table 1 shows that LILR increases the excimerization coefficient of pyrene probe F_E/F_M (282) by 55% ($p<0.01$) in the erythrocyte suspension, which attests to a decrease in the relative microviscosity of the protein-lipid contact regions. The excimerization coefficient of pyrene probe F_E/F_M (334) also decreased (by 25%, $p<0.05$), which indicates a decrease in the microviscosity of the lipid bilayer of the erythrocyte plasma membrane. A 22% decrease in dF parameter reflects a decrease in submersion of proteins into the lipid bilayer. The polarity of the environment of the hydrophobic probe pyrene, that diffuses into the region of fatty acid chains of membrane phospholipids, remains at the control level, which indicates the stability of the F372/393 fluorescence ratio.

Bearing in mind that LP is one of the most important factors affecting the viscoelastic properties as well as the protein-protein and lipid-protein interaction in cytoplasmic membranes [2], we studied the effect of LILR on the level of MDA, an LP terminal product. LILR did not change appreciably plasma MDA concentration and significantly decreased MDA content in erythrocytes (by 31%, Table 2). There was a minor increase in the activity of plasma ceruloplasmin caused by LILR. In addition, there was a

TABLE 1. Effect of Laser Radiation on the Parameters of Fluorescent Probe Pyrene in Erythrocyte Suspension (*M±m*)

Time of measurement	F_E/F_M (334)	F_E/F_M (282)	ΔF, rel. Units	F372/393
Initial data	0.44±0.05	1.77±0.11	1.65±0.10	1.02±0.01
Postradiation data	0.55±0.06*	2.74±0.17*	1.28±0.08*	1.03±0.001
Changes, Δ %	+25	+50	-25	0

Note. ΔF is an index of effectiveness of radiation-free transfer of energy of electron excitation from the tryptophan residues of membrane proteins to pyrene; F372/393 is the ratio of intensities of fluorescence from the maximum wavelengths 372 and 393 nm at the excitation wavelength of 334 nm. Here and in Table 2: *$p<0.05$ relative to initial data.

TABLE 2. Effect of Laser Radiation on MDA Level, the Content of Ceruloplasmin and Medium-Sized Molecules (*M±m*)

Time of measurement	MDA content		Plasma ceruloplasmin, μmol/liter	Concentration of medium-sized molecules, optical density units
	in plasma, nmol/ml	in erythrocytes, nmol/mg hemoglobin		
Initial data	6.52±0.48	2.78±0.34	2.19±0.41	0.29±0.05
Postradiation data	5.99±0.38	1.92±0.06*	2.68±0.42	0.21±0.01*

decrease in the plasma content of the medium-molecular-weight molecules.

According to modern views, laser radiation activates electrons and possibly induces their transition to other energy levels, which provides conditions for the activation of biochemical reactions. It is probable that the synthesis and conversion of the labile products of photobiological process, namely, free radicals, reduced and oxidized radical ions, is a short but a very important stage for subsequent events. This may account for the fact that LILR in our experiment neither activated LP nor increased the number of medium-molecular-weight molecules. The data point to a certain antioxidizing effect of LILR, which is consistent with the literature data [9]. Inhibition of LP is known to decrease the content of unsaturated lipids, which increases the rigidity of cell membrane. Thus, the observed changes in microviscosity cannot be ascribed to LP. It seems that the membranotropic effect of LILR is of primary nature.

Our data suggest the following scheme of LILR effect on blood cells: absorption of radiation quanta by protein molecules → increase in the protein energy with a concomitant local heating and conformation rearrangements → decrease in the lipid bilayer microviscosity and a tendency toward a decrease in the microviscosity in the lipid-protein contact regions → conformational changes in the membrane receptors and shifts in membrane potentials manifested in our experiments as a decrease in functional activity of the platelets → physiological effect manifested in suppression of platelet aggregation → amelioration of blood microcirculation.

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