Effect of Laser Irradiation on Functional Activity of Human Neutrophils: Activation of Myeloperoxidase in the Presence of Hematoporphyrin

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Laser irradiation (λ=540 nm) of the blood increased myeloperoxidase activity in polymorphonuclear leukocytes. We revealed photoinduced activation of myeloperoxidase in irradiated neutrophils in the presence of hematoporphyrin. The photodynamic effect was most pronounced, when the modifier was incorporated into the cell membrane or sorbed on it.

Key Words: *neutrophil; myeloperoxidase; laser irradiation; hematoporphyrin*

An important role in nonspecific organism's resistance is played by neutrophilic leukocytes. The granular apparatus of these cells possesses high bactericidal potential and is involved in the inactivation of phagocytized microorganisms. Myeloperoxidase (MPO, EC 1.11.1.7) is the major component of primary neutrophil granules possessing bactericidal properties and cleaving endogenous H_2O_2 [13]. Reduced MPO activity impairs organism's resistance to infectious agents and promotes pathological processes. Inflammation exhausts the antioxidant system, which leas to accumulation of reactive oxygen species [8,10]. The search for new methods directed at the maintenance of MPO activity in the blood is of considerable importance.

Low-intensity He-Ne laser irradiation (632.8 nm) is widely used in clinical practice for the therapy of various inflammatory diseases, including sepsis, peritonitis, purulent cholangitis, and chronic gastric ulcers [4]. Laser therapy improves microcirculation, coagulation and immune properties of the blood [4,6]. Irradiation with argon (488/514 nm) or Nd:YAG laser is used for the therapy of eye diseases [9,15], neurosurgical pathologies, [9,16], and virus-induced tumors [9,14].

The effects of laser irradiation on biological systems are due to the presence of acceptors that can absorb visible light. In the blood system these acceptors are presented by various forms of hemoglobin, catalase, cytochrome *b*, cytochrome oxidase, and peroxidase. Here we studied the effect of irradiation with an yttrium-aluminum laser (540 nm) on functional activity of human blood polymorphonuclear leukocytes (PMNL).

MATERIALS AND METHODS

PMNL were isolated by centrifugation of donor blood in a Ficoll-Urografin double-density gradient (ρ=1.077 and 1.119 g/ml) [7] and lyzed for preparing MPO-containing supernatant (2 ml distilled water per 1 ml suspension). NaCl (0.6 ml, 3%) was added after 30 min, and the mixture was centrifuged at 3000 rpm for 30 min.

A neodymium:yttrium-aluminum-garnet laser was used. The intensity of laser irradiation was measured using an IMO-24 device. PMNL suspension in 3 ml Hank's solution (5×10^5 cells/ml) and heparinized blood (3 ml, 50 U heparin) were irradiated in a thermostatic cuvette under constant mixing (0.43 mW dose rate, 0.7 cm spot diameter, 0.35-4.20 J/cm^3 dose range, 20±1°C).

MPO activity was measured by the method described elsewhere [2]. The reaction mixture contained 0.5 ml hemolysate. The substrate mixture was prepared using phosphate-citrate buffer (pH 6.0). The reac-

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tion was conducted for 10 min. The content of *o*-dianisidine (oxidation product) was measured on a Multiscan Plus multichannel spectrophotometer at λ=492 nm using molar extinction coefficient ε=1.78×10²/mol/cm.

Extracellular MPO activity was estimated by inhibition of phagocytosis using charged flat-bottom plates for enzyme immunoassay. The cell suspension (50 μl) was placed in wells and incubated for 2-45 min. The substrate mixture (100 μl) containing 3.7×10^{-3} mol/liter *o*-phenylenediamine and 4×10^{-3} mol/liter H_2O_2 in Hank's solution was added for 5 min.

Hematoporphyrin (HP, Soret band at λ_{max}=387 nm, ε=48×10³/mol/cm) [3] was obtained from human hemoglobin as described elsewhere [1].

The results were analyzed by pairwise Student's *t* test (Statgraphics software). The differences were significant at $p<0.05$.

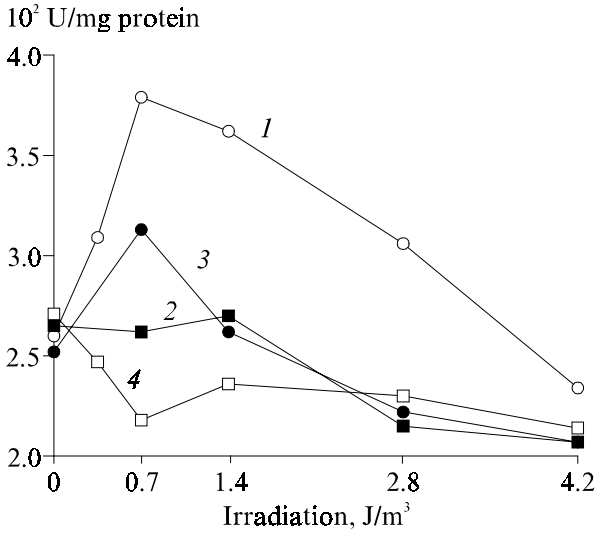

Fig. 1. Effect of laser irradiation of the blood and neutrophil suspension on myeloperoxidase activity in neutrophils of irradiated blood (*1*) and irradiated neutrophils (*2-4*).

RESULTS

Figure 1 shows the dependence of MPO activity on the dose of laser irradiation (LI) of the blood. Irradiation with 0.35-1.40 J/cm³ increased enzyme activity. This effect was most pronounced after irradiation in a dose of 0.7 J/cm³ (46% increase in MPO activity). Increasing the dose of LI to 4.2 J/cm³ led to inhibition of enzyme activity (Fig. 1).

When studying activity of extracellular MPO released from azurophilic granules during phagocytosis, we found that MPO activity in native PMNL was maximum after 10-min incubation (Fig. 2). MPO activity was above the control after LI of the blood with 0.7 J/cm³. After 10-min incubation, enzyme activity in PMNL increased by 42%. Increasing the time of incubation led to a decrease in MPO activity, especially in PMNL from irradiated blood.

Previous studies showed that phagocytosis is accompanied by respiratory burst [8]. After interaction with large objects that can not be phagocytized, neutrophils release reactive oxygen species (ROS) that are probably involved in MPO destruction. Their content can be evaluated by the contribution of oxidants in enzyme inactivation. LI of the blood probably intensifies ROS generation by neutrophils.

LI of the blood increased MPO activity in all donors. These donors were divided into 3 subgroups depending on the effects of LI on PMNL resuspended in Hank's solution (Fig. 1). In group 1 donors, irradiation of PMNL with 0.35-1.40 J/cm³ produced no dose-dependent changes in MPO activity (curve *2*). In group 2 donors, enzyme activity increased by 24% after irradiation with 0.7 J/cm³ (curve *3*). LI with 4.2 J/cm³ decreased MPO activity in group 1 and 2 donors by 22 and 18%, respectively. In group 3 donors, irradiation with 0.7-4.2 J/cm³ inhibited enzyme activity effects (curve *4*). Further experiments were performed with group 1 donors irradiated with 0.7 J/cm³.

Porphyrins, whose content increases during various diseases, can act as primary chromophores of laser radiation in the visible region of the spectrum [5]. We studied the effect of LI on MPO activity in neutrophils in the presence of exogenous HP. HP in concentrations of $1\text{-}8\times10^{-16}$ mol/liter did not change MPO activity in the PMNL suspension. Increasing the concentration of HP to 10^{-14} and 8×10^{-11} mol/liter decreased enzyme activity by 77 and 64%, respectively. LI of PMNL in the presence of HP increased MPO activity (Table 1). Enzyme photosensitivity increased with an increase in the concentration of HP in the range of $1\text{-}8\times10^{-16}$ mol/liter. In the presence of HP in concentrations of 2×10^{-16}, 4×10^{-16}, and 8×10^{-16} mol/liter, LI of PMNL increased MPO activity by 53, 81, and 85%, respectively.

The mechanisms of HP-induced photosensitizing effects on cells include various physical and chemical processes: absorption of a light quantum by the mo-

TABLE 1. Effect of Laser Beam on MPO Activity in Irradiated Neutrophils (0.7 J/m³) in the Presence of HP (*M*±*m*)

HP concentration, 10^{-16} mol/liter	Activity, %	
	in the presence of HP	washed cells
1	122±6	—
2	153±5	125±6
4	181±5	139±6
8	185±5	156±5

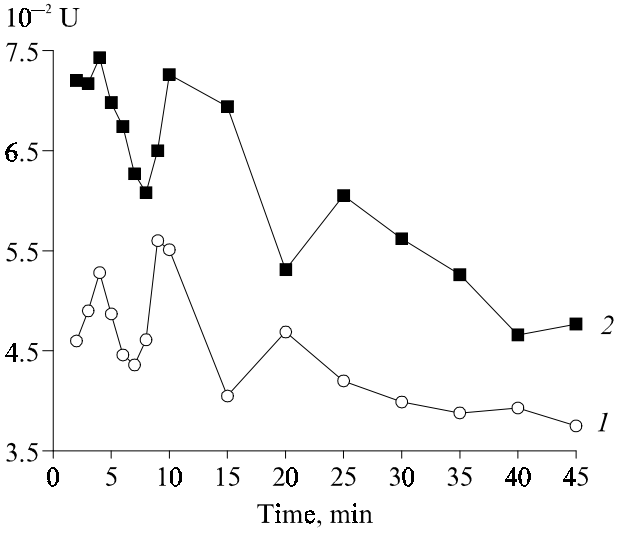

Fig. 2. Effect of laser irradiation of the blood on extracellular myeloperoxidase activity during inhibition of phagocytosis: before (*1*) and after irradiation (*2*, 0.7 J/cm^3).

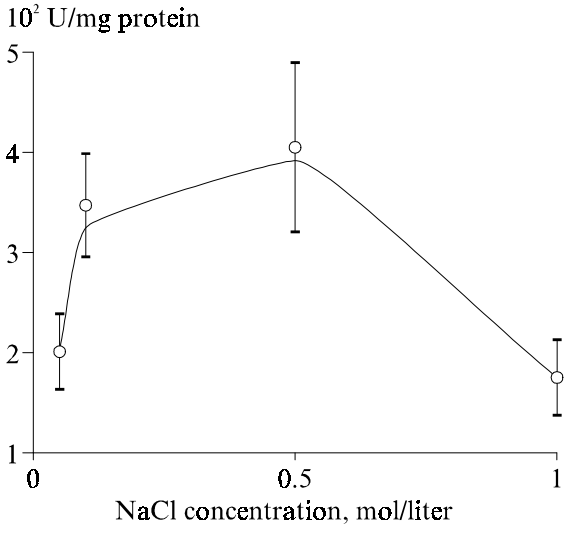

Fig. 3. Myeloperoxidase activity in the presence of NaCl.

difier molecule and its excitation, generation of $^1O_2$ by energy transfer from excited HP molecules to O_2 [11], and chemical transformation of biological structures. Water-soluble HP intensively generates singlet oxygen in water solutions (quantum yield for HP is 0.48) [17].

Chlorides serve as MPO cofactors during oxidation of various substrates in the presence of H_2O_2 [10]. Our results indicate that increasing the concentration of NaCl in the supernatant from 0.09 to 0.5 mol/liter led to MPO activation by 31% (Fig. 3). The peroxidase reaction was inhibited at high concentration of NaCl (0.7 mol/liter). Photodynamic effects related to the presence of sensitizing agents probably increase cell membrane permeability. Therefore, the influx of Cl^- can modulate MPO activity. Since double bonds in carbohydrate chains serve as the target for phototoxic effects of HP, photodestruction would be most pronounced if the modifier is localized in the cell membrane.

We evaluated the contribution of HP molecules sorbed to or localized in the cell membrane to LI-induced changes in MPO activity. PMNL were incubated with 2×10^{-16}, 4×10^{-16}, and 8×10^{-16} mol/liter HP for 40 min and washed 2 times with Hank's solution. LI of washed cells increased MPO activity by 25, 39, and 56%, respectively, which was 30-40% below this parameter in irradiated PMNL before removal of free HP molecules from the cell suspension (Table 1). Probably, both solubilized and sorbed HP molecules play a role in photoinduced changes in MPO activity in irradiated cells. It cannot be excluded that green light-sensitive porphyrin is bound to neutrophil membranes. The chromophoric group of HP transduces electronic excitation energy to membrane components. Thermal energy produced in nonradiative energy transfer causes local heating of the photoreceptor, which promotes its reorientation. Porphyrin undergoes intermediate relaxation stages contributing to dynamic and static conformational changes in the membrane, to which HP is bound, and variations of the membrane potential.

Thus, structural and functional reconstructions of cell membranes and intracellular organelles underlie the effect of LI on blood neutrophils. This is probably associated with the resonance absorption of 540-nm light by specific light acceptors and the development of oscillatory excitation, which modulates lipid peroxidation and leads to local or total conformational changes in membranes. These processes provide a physicochemical basis for cell reactions, including changes in ion permeability, activation of MPO due to energy transfer from excited HP molecules sorbed on the inner membrane (HP crosses the membrane and is sorbed on its external and internal layers [12]), and photoactivation of MPO after LI of the enzyme molecule. The interactions between blood cells can also mediate LI-induced changes, *e.g.*, via activation of lymphokine-producing lymphocytes [8].

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