Modification of Membrane-Toxic Effect of Nd-YAG Laser by Preliminary Induction of Cytochrome P-450 in Bone Marrow Cells

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The cytotoxic effects of Nd-YAG laser with a wavelength of 533 nm were studied in mouse bone marrow cells *in vitro.* Laser exposure caused blebbing of bone marrow cell plasma membrane. Preliminary induction of cytochrome P-450 with 3-methylcholanthrene modified the cytotoxic effect of Nd-YAG laser.

Key Words: *bone marrow; blebbing; Nd-YAG laser; cytochrome P-450*

Laser-induced photoprocesses play a role in the regulation of functional activity of biological structures in a live organism, but the mechanism of these processes is still unclear. Photosensitizes (molecules absorbing light and inducing reactions which do not occur in their absence) are essential for the realization of effects induced by lasers [1,2]. Light absorption causes transition of these molecules into the exited state, which ensures realization of photoeffects. The photoactive chromophore molecule modifies the state of its microenvironment. These changes induce further structural and functional rearrangements triggering biochemical processes and culminating in a final biological effect [4].

Nd-YAG laser with a wavelength of 533 nm can induce photoprocesses in hemoproteins [2]. Cytochromes P-450, photosensitive hemoproteins, catalyze hydroxylation of lipophilic compounds (by products of metabolic reactions or exogenous substances). Bone marrow cells (BMC) express cytochrome P-450 IA1. It participates in the regulation of proliferation of hemopoietic cell via oxidation of peptide products of cell metabolism, regulation of cell content of bioactive substances, and regulation of the arachidonic acid cascade [6]. Free radicals formed during cytochrome P-450-catalyzed oxidation can damage cell structures [3]. Blebbing is a manifestation of the toxic effect of free radicals on cell membranes. The mechanisms of blebbing are still poorly understood. It is believed that impaired structure of backbone proteins, activity of nonlysosomal enzyme systems, and ionic imbalance play an important role in this process.

Here we evaluated the type and mechanisms of membrane-toxic effects of Nd-YAG laser on mouse BMC *in vitro.*

MATERIALS AND METHODS

Random-bred male albino mice weighing 20-25 g were used (5 animals per series).

Blebbing of plasma membrane was studied by phase-contrast microscopy: 200 cells in each preparation were analyzed under immersion objective (×900). The samples were exposed to Nd—YAG laser (λ=533 nm, power density 200 mW/cm^2) during 5, 10, and 15 min in the flash photolysis mode (cell concentration 10^6/ml in Hanks solution) at 20°C. Small peripheral bubble-like protrusions (initial blebbing), larger bubbles (terminal blebbing), and necrosis foci were counted.

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The content of malonic dialdehyde (MDA) in BMC was measured by the reaction with thiobarbituric acid (TBA). Optical density was measured on an SF-26 spectrophotometer at 535 nm against butanol. MDA content was estimated using molar extinction coefficient.

3-Methylcholanthrene was injected intraperitoneally in a dose of 50 mg/kg once a day for 3 days.

The results were statistically processed using Student's *t* test.

RESULTS

Exposure to Nd—YAG laser significantly reduced the number of morphologically intact cells ($p<0.01$). The number of cells with signs of initial or terminal blebbing and necrotic cells significantly increased in comparison with the control ($p<0.05$). Similar effects were observed in animals injected with 3-methylcholanthrene, but after irradiation the number of morphologically intact cells in suspensions isolated from these animals decreased to a lesser extent. Accumulation of cells with initial blebbing and necrosis was less pronounced, while the percentage of cells with signs of terminal blebbing significantly increased ($p<0.01$, Fig. 1).

MDA production under the effect of laser exposure was suppressed in cells with induced cytochrome P-450, but not in the control ($p<0.01$, Fig. 2).

Hence, irradiation with Nd—YAG laser (λ=533 nm) induced blebbing in the plasma membrane of BMC *in vitro* and stimulates LPO in membranes of these cells. Preliminary injection of cytochrome P-450 inductor considerably modified these effects.

Direct and free radical-mediated proteotoxic effects of laser irradiation are well known [2]. It can be

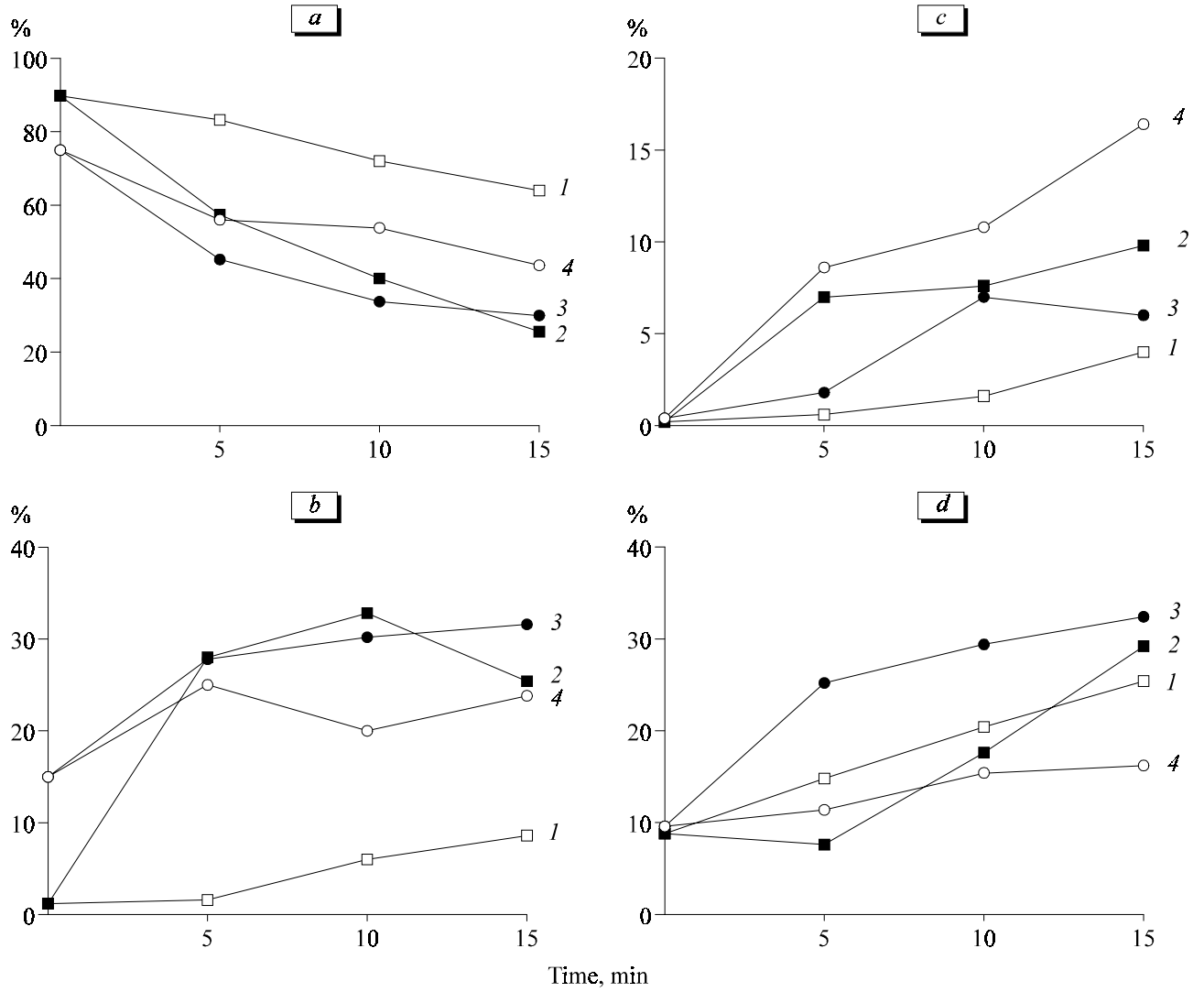

Fig. 1. Morphological changes in bone marrow cell *in vitro* in the control (*1*), under the effect of Nd—YAG laser (λ=533 nm) (*2*), after induction of cytochrome P-450 with 3-methylcholanthrene (*3*), and after a combination of both factors (*4*). *a*) morphologically intact cells; *b*) cells with initial blebbing; *c*) cells with terminal blebbing; *d*) necrotic cells. Here and in Fig. 2: the initial level corresponds to zero on the abscissa.

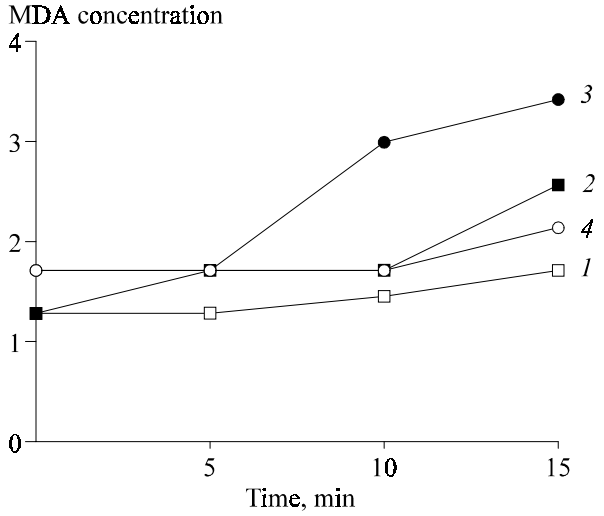

Fig. 2. LPO intensity in mouse bone marrow cells *in vitro* in the control (*1*), under the effect of Nd—YAG laser (*2*), after induction of cytochrome P-450 with 3-methylcholanthrene (*3*), and after a combination of both factors (*4*).

assumed that conformation rearrangements of membrane proteins and cytoskeleton induced by Nd—YAG laser are involved in the pathogenesis of blebbing.

Induction of cytochrome P-450 IA1 with 3-methylcholanthrene stimulated production of free radicals, which, as was shown for other cell systems [4], damage backbone proteins and lead to the development of ionic imbalance and activation of nonlysosomal proteolytic systems. Interestingly, laser exposure after induction of cytochrome P-450 IA1 with 3-methylcholanthrene was accompanied by less pronounced initial blebbing and necrotic processes, while the number of BMC with signs of terminal blebbing increased. We hypothesize that induction of cytochrome P-450 is associated with enhanced formation of "targets" for laser due to accumulation of newly synthesized hemoproteins. Photodynamic processes inactivate cytochrome P-450, thus suppressing generation of free radicals. Hence, the effects of oxidative stress in these cells are less pronounced. On the other hand, photoinduced processes in transmembrane and membrane-associated proteins do not disrupt peptide bonds in the proteins; excitation of fluctuations and rotations is superimposed on the ground electron excitation due to the presence of aromatic amino acids [4,7] and the energy of electron excitation leads to conformation rearrangements in protein molecules, thus modifying their sensitivity to proteotoxic and membrane-toxic factors.

Hence, induction of cytochrome P-450 can modify morphological changes in BMC membrane and membrane LPO caused by laser exposure.

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