EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Effect of Laser Radiation on Production of Reactive Oxygen Species in the Blood of Patients with Chronic Obstructive Pulmonary Disease

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The effect of laser radiation on generation of reactive oxygen species in the whole blood from patients with chronic obstructive pulmonary disease was studied by *in vitro* recording of luminol-dependent chemiluminescence. Laser irradiation of the blood from patients with increased production of reactive oxygen species decreased the microbicidal potential of cells. In patients with low generation of reactive oxygen species and normal potential of cells, laser exposure increased production of O_2 metabolites. Laser radiation had little effect on chemiluminescence of the blood in patients with low generation of reactive oxygen species and decreased functional activity of cells.

Key Words: *chemiluminescence; reactive oxygen species; laser blood irradiation*

Laser irradiation of the blood (LIB) is a promising method of therapy in patients with nonspecific lung diseases [1,3]. The therapeutic effect of LIB is associated with activation of phagocytizing cells and stimulation in the production of reactive oxygen species (ROS), NO, and cytokines [2,5,10]. LIB can decrease the content of lipid peroxidation products, medium-molecular-weight molecules, and circulating immune complexes in the blood of patients with nonspecific lung diseases [4,7]. In clinical practice, empirical decisions are often made to introduce laser therapy into the scheme of patient's treatment. The effects of laser radiation on cell function in patients with lung diseases are poorly understood and indications for LIB are unknown.

Here we studied the *in vitro* effect of LIB on ROS generation in whole blood from patients with chronic obstructive pulmonary disease.

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MATERIALS AND METHODS

Experiments were performed on the whole blood from 16 patients with chronic obstructive pulmonary disease. Two blood samples from the cubital vein (5 ml) were taken from fasting individuals in the morning into plastic tubes with heparin (50 U/ml). The tubes were immediately put in a dark chamber at 37°C. The end of a light guide of an ALTO-500/4-06 semiconductor laser oscillator (wavelength 0.67 μ, Al'to) was immersed in one blood sample to deliver continuous LIB. Laser power at the end of the light guide was 2 mW, the exposure was 1 min, the energy emitted during LIB was 0.12 J/cm^2.

Another blood sample from the same patient was used as the control (dark incubation).

ROS generation in the blood sample subjected to LIB or maintained under control conditions was studied by recording spontaneous and luminol-depended chemiluminescence (CL_S and CL_I, respec-

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tively) on a Khemilyuminomer-003 device (UGATU) [9]. CL_S of the whole blood was measured over 5 min. The blood (0.1 ml) was added to 2 ml physiological saline with 10^{-5} M luminol and put in a temperature-controlled chamber (37°C). CL_I reflected microbicidal potential of cells. We used an 18-h-old culture of *Staphylococcus* strain 209 (10^9 microbial cells per ml). The blood (0.1 ml) was mixed with 0.01 ml suspension of staphylococcus culture in an immunological plate, incubated for 2 min, and the mixture was added to 2 ml physiological saline with luminol. Induced chemiluminescence was measured for 5 min. ROS generation in the blood from patients was estimated by total CL yield. The results were expressed in relative units. We calculated the ratio of estimated chemiluminescence to standard chemiluminescence (total light flux 5.1×10^5 quanta per sec). CL of the blood from 20 conventionally healthy donors was measured in a comparative study.

The results were analyzed by Student's *t* test.

RESULTS

The patients were divided into 3 groups by CL of control samples from the whole blood and change in CL after LIB. Total CL_S and CL_I of the whole blood from group 1 patients (n=5) were higher than in healthy donors (by 1.9 and 1.8 times, respectively; Fig. 1, *a*), which attests increased production of ROS in blood samples. LIB had little effect on CL_S in these patients. It can be hypothesized that blood samples activated before the start of LIB exhibited a weak response to laser radiation. Moreover, total CL_I of blood samples reflecting the reserve capacity of phagocytizing cells from these patients decreased by 2.3 times after exposure to laser radiation.

Total CL_S in group 2 patients (n=6) was 1.4-fold lower than in healthy donors, which reflected decreased ROS generation in the blood (Fig. 1, *b*), but total yield of CL_I of the blood was high. Hence, functional reserve of blood cells was preserved in

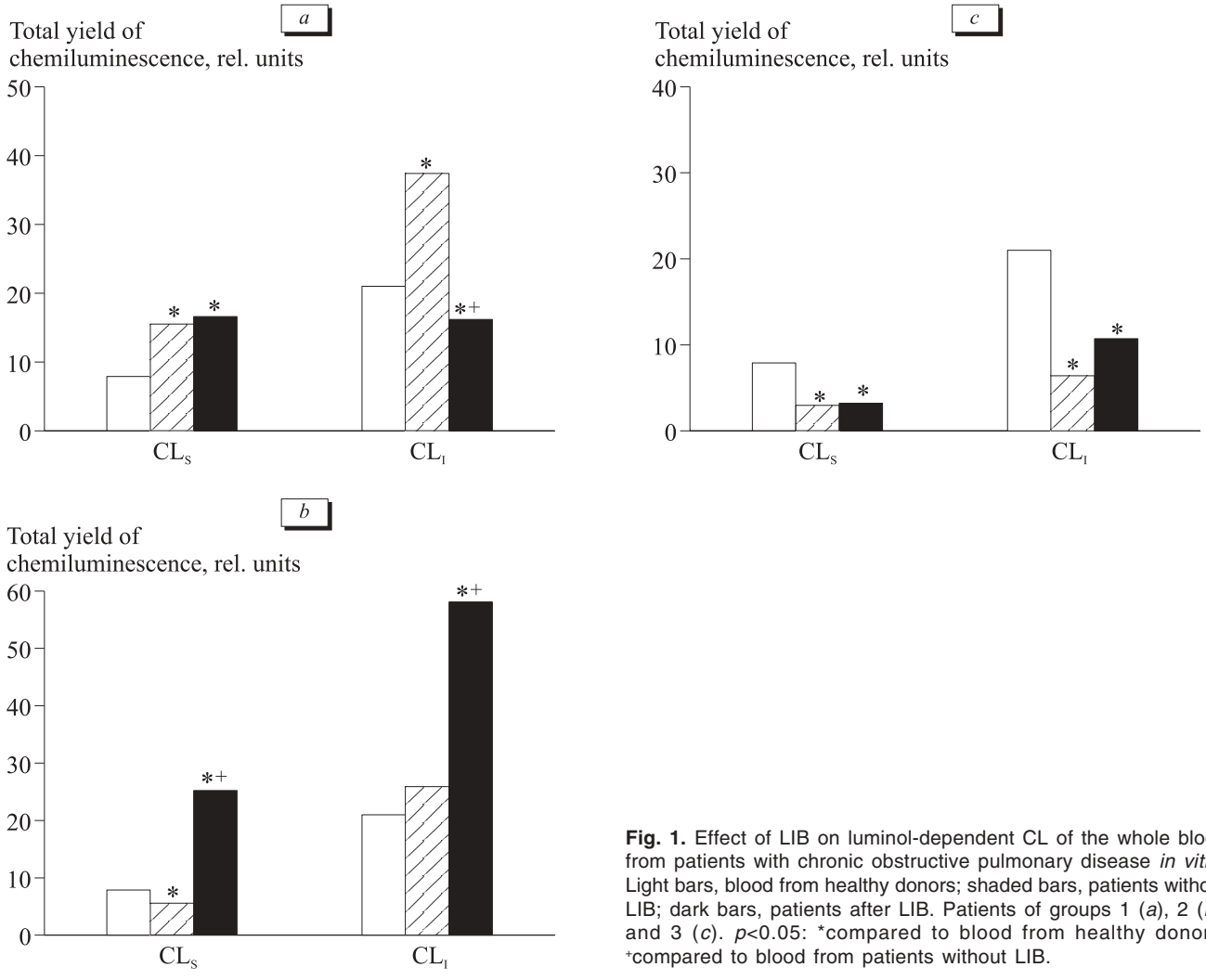

Fig. 1. Effect of LIB on luminol-dependent CL of the whole blood from patients with chronic obstructive pulmonary disease *in vitro*. Light bars, blood from healthy donors; shaded bars, patients without LIB; dark bars, patients after LIB. Patients of groups 1 (*a*), 2 (*b*), and 3 (*c*). $p<0.05$: *compared to blood from healthy donors; +compared to blood from patients without LIB.

these patients. LIB increased CL_S and CL_I in these patients by 4.5 and 2.2 times, respectively. Therefore, normal microbicidal potential of blood cells provides their activation by laser radiation.

Total CL_S and CL_I in group 3 patients (n=5) were lower than in healthy donors by 3.3 and 2.7 times, respectively (Fig. 1, *c*). These changes reflect functional suppression of blood cells. LIB did not significantly increased CL_S and CL_I in the blood from these patients. Low functional potential of cells probably contributes to the reduced response to laser radiation.

Studying of the effect of LIB on blood cells should take into account the fact that laser radiation can modulate some general processes, including free radical oxidation. Laser treatment potentiates the formation of singlet O_2 and other ROS, which is mediated by endogenous porphyrins [10]. This effect of laser radiation on cells not only primes, but also decreases phagocytosis under certain conditions [6,8]. Previous studies showed that LIB decreases the microbicidal potential of blood cells during ROS overproduction. When ROS generation in the blood was suppressed, the cell response to LIB depended on the functional reserve of these cells. Low potential of cells was characterized by a decrease in CL_S and CL_I of the blood and inhibition of the response to LIB. By contrast, LIB contributed to the increase in microbicidal activity of cells with normal potential. Hence, the directionality of LIB-induced changes in the production of O_2 metabolites depends on functional activity of cells subjected to laser radiation. Taking into account these data, we conclude that luminol-dependent CL of the whole blood can serve as a criterion for the use of LIB in patients with lung diseases.

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