Effect of Low-Energy Laser Irradiation on the Area of Experimental Myocardial Infarction, Lipid Peroxidation, and Hemoglobin Affinity for Oxygen

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The mechanism of antiischemic effect of low-energy laser radiation was studied in rats with experimental myocardial infarction taking into consideration the effect of laser on hemoglobin affinity for oxygen and intensity of lipid peroxidation. Low-energy laser irradiation for 15 min *in vitro* decreased the area of myocardial infarction, slightly reduced hemoglobin affinity for oxygen, and inhibited lipid peroxidation. Short-term low-energy laser irradiation did not reduce the area of necrosis, initiated lipid peroxidation, and increased superoxide dismutase activity.

Key Words: *antiischemic effect; hemoglobin affinity for oxygen; lipid peroxidation; low-energy laser radiation*

Low-energy laser radiation (LELI) was extensively used in the therapy of patients with coronary heart disease over the last 2 decades [6,8]. For more detailed investigation of the mechanisms underlying the cardioprotective effect of LELI it is important to evaluate *in vitro* changes in hemoglobin affinity for oxygen (HAO) after LELI therapy in doses producing the antiischemic effect. The increase in hemoglobin P_{50} by 2.0-4.2 mm Hg promotes O_2 release and increases O_2 supply to tissues by 20-30% [12]. Previous studies showed that LELI improves oxygen transport by erythrocytes and decreases HAO [9]. Published data show that synthetic allosteric stimulators of oxyhemoglobin dissociation possess antiischemic activity [11]. These data accentuate the importance of the studied problem.

Here we studied *in vitro* effect of LELI on HAO in rabbit blood and area of experimental myocardial infarction in rats. Since therapeutic effect of LELI during ischemic injury [3] is associated with changes in the intensity of lipid peroxidation (LPO), we measured some parameters of the prooxidant-antioxidant state.

MATERIALS AND METHODS

Experiments were performed on male and female rats weighing 200-250 g. Continuous radiation was delivered using a Kreolka-31 semiconductor laser (Tekhnika-Pro, λ=630 nm, 3 mW) placed at a distance of 4 cm from the irradiated surface. The beam was directed perpendicularly to the irradiated object (rabbit and human blood, precordial area of rat thorax). The time of exposure was 2 and 15 min (0.018 and 0.138 J/cm^2, respectively).

HAO was measured in blood samples from the right atrium of rabbits. The test preparation dissolved in 0.1 ml isotonic NaCl was added to 5 ml heparinized blood and incubated at 37°C for 120 min. HAO was determined by P_{50} (method of mixing) [3]. Two portions of the sample (1.5-2.0 ml) were exposed to maximum oxygenation and deoxygenation. P_{50} was assayed in 2 equal samples on an ABL-330 Radiometer gas microanalyzer.

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The area of myocardial infarction in rats was estimated gravimetrically and expressed in percents of the weight of the left ventricle. Examination was performed 4 h after ligation of the anterior descending branch of the left coronary artery at a level of the lower edge of the left auricle.

The total yield of Fe^{2+}-induced chemiluminescence was recorded on a BKhL-06 biochemiluminometer. The plasma was obtained from rats (200-250 g) before and 4 h after coronary artery ligation. The content of malonic dialdehyde (MDA) in plasma samples was measured.

Accumulation of MDA and activity of Cu,Zn-superoxide dismutase (SOD) were *in vitro* studied in blood hemolysates. The amount of SOD decreasing the rate of cytochrome *c* reduction in the xanthine—xanthine oxidase system by 50% was taken as a unit of enzyme activity [13]. MDA concentration was measured in the reaction with thiobarbituric acid [10]. The system of NADPH-dependent LPO contained LPO-activating agents $FeSO_4$ and NADPH in concentrations of 1.2 and 0.77 μM, respectively. Changes in MDA content and SOD activity were recorded immediately after LELI.

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

RESULTS

LELI for 15 min reduced the area of experimental myocardial infarction 4 h after coronary artery ligation (Table 1). These changes were accompanied by a significant reduction of MDA accumulation and inhibition of Fe^{2+}-induced chemiluminescence (Table 1). Our results are consistent with published data that inhibition of LPO serves as a criterion for adequate LELI therapy [3].

LELI for 15 min *in vitro* slightly decreased HAO, which was followed by an increase in P_{50} (Table 2). We revealed a rightward shift of oxyhemoglobin dissociation curve (statistically insignificant), which reflected the increase in O_2 release. These changes are associated with improvement of oxygen balance in hypoxic tissues. This effect of LELI probably contributes to a decrease in the area of injury in rats with acute stage of myocardial infarction. LELI increases the rate of electron transport in the mitochondrial respiratory chain, which can improve functions of the ischemic myocardium [4].

The dynamics of MDA accumulation *in vitro* showed that 2-min LELI markedly stimulates LPO and induces a compensatory increase in SOD activity (Table 2). Similar changes in erythrocyte SOD activity were observed previously in clinical trials. The intensity of MDA accumulation and SOD activity decreased after 15-min LELI (Table 2).

TABLE 1. Effect of LELI on the Area of Injury and Indexes of LPO in Rats with Experimental Myocardial Infarction (*M*±*m*)

Group	Area of myocardial infarction, % of the weight of the left ventricle	MDA, μmol/liter			Fe^{2+}-induced chemiluminescence, arb. units		
		initial state	after 4 h	shift, %	initial state	after 4 h	shift, %
Control (*n*=10)	47.38±0.45	0.405±0.006	0.644±0.011	+59.35±0.39	0.450±0.006	0.841±0.014*	+86.88±0.22
LELI 2 min (*n*=16)	45.69±0.76	0.404±0.004	0.605±0.004	+50.56±0.10	0.431±0.007	0.814±0.014	+89.91±0.27
15 min (*n*=16)	37.81±1.09*	0.408±0.006	0.590±0.008*	+44.83±0.13	0.436±0.007	0.796±0.016*	+83.28±0.25

Note. Here and in Table 2: *$p<0.05$ compared to the control.

TABLE 2. *In Vitro* Effects of LELI on HAO and LPO (*M*±*m*)

Group		P_{50}, mm Hg	MDA, nmol/g Hb	SOD, U/g Hb
Control		30.57±0.35 (*n*=8)	1.5±0.1 (*n*=10)	1555±41 (*n*=10)
LELI	2 min	30.91±0.72 (*n*=5)	4.40±0.18* (*n*=10)	2247±55* (*n*=10)
	15 min	32.02±1.02 (*n*=8)	2.20±0.17 (*n*=10)	1846±37* (*n*=10)

Our results show that 15-min LELI reduces the area of myocardial infarction in rats 4 h after coronary artery ligation. This treatment slightly decreased HAO, which improves oxygenation in ischemic tissues. LELI abolishes accumulation of MDA and decreases Fe^{2+}-induced chemiluminescence in rat plasma 4 h after coronary artery ligation. Short-term LELI (2 min) has no effect on the area of myocardial infarction, activates *in vivo* and *in vitro* LPO, and induces a compensatory increase in SOD activity.

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