EFFECT OF LOW-INTENSITY LASER RADIATION ON SOME METABOLIC PARAMETERS OF THE BLOOD AFTER RESUSCITATION

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Laser therapy, using monochromatic radiation of a helium–neon laser with wavelength of 632.8 nm and a power density of several milliwatts per square centimeter has been used in various pathological states. The efficacy of the laser in hypoxia has been demonstrated by experimental and clinical investigations.

Two fundamental hypotheses on the mechanisms of the biological action of radiation of the helium–neon laser are currently being discussed. According to the first, the effect is due to structural changes in the irradiated liquid medium with a change in its physical properties, including its ability to dissolve oxygen [4, 8]. The second is based on the fact that the spectrum of biological action of the laser coincides in great detail with the spectrum of photoexcitation of singlet oxygen [1]. As a result, induction of enzyme systems such as superoxide dismutase [2] and catalase [3] may take place in the tissues. Ideas have developed according to which components of the respiratory chain are the primary photoacceptor [5].

Information on the mechanisms of action of the laser in hypoxia and, in particular, in the postresuscitation period is fragmentary and vague in character, and this accounts for the urgent importance of the present study.

EXPERIMENTAL METHOD

Experiments were carried out in vitro with whole canine blood taken after 15 min of the postresuscitation period following recovery of the animal from hemorrhagic shock. To ensure uniform irradiation of the whole blood volume (50 ml) oxygen was bubbled through it in an oxygenator for 5 min, with a gas flow rate of 1 liter/min. The power density of laser radiation (IHNL) was 0.3 mW/cm^2. The partial pressure of oxygen (pO_2) and carbon dioxide (pCO_2) was determined on a "Rodelkis" gas microanalyzer. Concentrations of bound and dissolved oxygen were calculated; the buffer base (BB) level and the buffer base deficit (BE) were determined from Siggard–Andersen nomograms. The fatty-acid composition of total blood lipids was determined by gas–liquid chromatography followed by calculation of the average length of the fatty acid chain and the number of double bonds [11]. Activity of lipid peroxidation (LPO) was estimated from the concentration of conjugated dienes (CD) [7]; malonic dialdehyde (MDA) as in [15]; Schiff bases (SB) as in [13]; total antioxidative activity (AOA) of the plasma as in [10], superoxide dismutase (SOD) activity of the erythrocytes as in [14], and their catalase activity as in [12]. The microviscosity of the erythrocyte membranes was measured in relation to excimerization of a fluorescent pyrene probe, using the "Signe-4" spectrofluorometer.

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TABLE 1. Effect of Oxygenation and Radiation of Helium–Neon Laser on Acid–Base State and Gas Composition of Blood in Postresuscitation Period (M ± m)

Blood sample	Parameters						
	pO_2, kPa	pCO_2, kPa	bound O_2, vol. %	dissolved O_2, vol. %	BB, μm	BE, μm	pH
Intact blood	13,3 ±1,3	2,20 ±0,13	19,36 ±0,99	0,302 ±0,03	49,38 ±1,15	—5,85 ±1,54	7,311 ±0,03
Blood in postresuscitation period	14,9 ±1,27	2,60 ±0,10	17,11 ±1,35	0,304 ±0,09	37,75 ±2,83[a]	—11,33 ±0,39[a]	7,236 ±0,025
Blood in postresuscitation period + O_2	23,7 ±0.9[a,б]	2,46 ±0,2	20,7 ±0,96	0,590 ±0,07[a,б]	38,00 ±2,08[a]	—9,88 ±1,28	7,410 ±0,033[б]
Blood in postresuscitation period + O_2 + IHNL	32,9 ±3,0[a,б,в]	2,20 ±0,2	20,30 ±1,015	0,743 ±0,07[a,б,в]	35,06 ±2,06[a]	—12,97 ±2,12[a]	7,434 ±0,033[б]

Legend. Here and in Table 2: a) significance of differences compared with intact blood, b) significance of differences compared with blood in postresuscitation period, c) significance of differences compared with oxygenation ($p < 0.001$).

TABLE 2. Effect of Oxygenation and Radiation of Helium–Neon Laser on State of Lipid Peroxidation in Postresuscitation Period (M ± m)

Parameters of LPO	Blood sample			
	intact blood	postresuscitation blood	postresuscitation blood + O_2	postresuscitation blood + O_2 + IHNL
CD	66,4±11,8	60,8±9,0	56,9±9,3	60,9±8,9
MDA	1,98±0,21	2,11±0,21	1,93±0,21	1,86±0,15
SB, relative units/ml	41,8±9,6	39,5±7,7	46,3±8,3	43,3±7,1
Total AOA, relative units	0,611±0,008	0,413±0,004[a]	0,503±0,012[a,б]	0,596±0,006[a,б]
SOD = conventional units / mg protein·min	14,36±1,79	8,12±0,91[a]	10,51±2,01	10,04±1,82[б]
Catalase μmoles H_2O_2 / ml·min	0,685±0,17	0,357±0,14	0,401±0,11[a]	0,725±0,13[a,б]

The results were subjected to statistical analysis and also to multiple linear regression analysis, using dialog regression programs and the CM-4 computer.

EXPERIMENTAL RESULTS

The period of the first 10-20 min after exposure to hypoxia is an early stage of recovery of the functions of the body from the beginning of resuscitation [9], when basically those disturbances that developed during hemorrhagic shock are preserved. This was confirmed by our analysis of the blood in the postresuscitation period [6], in which, despite restoration of the gas composition, pH and BB remained low, with the highest possible BE, as well as a high lactate level; AOA was significantly reduced but the intensity of LPO did not differ from that in intact animals.

During oxygenation of blood in the postresuscitation period the partial pressure of oxygen (pO_2) in it rose by 85%, whereas during combined treatment with IHNL it rose by 120% (Table 1). The bound O_2 showed no significant change whereas dissolved O_2 increased when oxygen was bubbled through by 85%, and when this was used in conjunction with the laser, by 144%. In both cases pH clearly shifted toward the alkaline side. The average length of the fatty acid chain remained unchanged. The number of double bonds was reduced by oxygenation, but under the

influence of the laser, on the contrary, it returned to normal. Despite the presence of an LPO substrate, namely polyunsaturated fatty acids and a significant quantity of oxygen, activation of LPO by IHNL was not observed (Table 2). The mean levels of molecular LPO products were unchanged compared with blood in the postresuscitation period. Meanwhile the reduced plasma AOA and the SOD and catalase activity of the erythrocytes returned to normal. Isolated oxygenation had no such effect.

After 5 min of oxygenation the viscosity of the erythrocyte membranes from blood in the postresuscitation period increased by 52%. Additional laser irradiation restored this parameter to its initial level. Membrane viscosity correlated negatively with SOD activity ($r = -0.701$) and with catalase activity ($r = -0.861$). Restoration of the membrane viscosity helped to restore the normal rheologic properties of the erythrocytes.

The results thus demonstrated definite shifts in a number of metabolic parameters of the blood in response to laser action, which differed from those obtained by oxygenation.

The absence of differences in the mean levels of LPO does not rule out the possibility of individual changes in the parameters in separate blood samples in response to IHNL. The following regular relationships between the changes in these parameters were obtained by means of multiple regression analysis:

$$\Delta_{\text{CD Las}} = -5{,}07 + 1{,}88\Delta\text{BB P}, \tag{1}$$

$$\Delta_{\text{SB Las}} = 16{,}058 - 10{,}065\Delta_{\text{Lac P}} + 59{,}04\Delta_{\text{Las}} \tag{2}$$

$$\Delta\text{AOA}_{\text{Las}} = 0{,}204 + 0{,}047\Delta O_{2\,\text{dissol.}}{}_{\text{Las}} \tag{3}$$

According to equation 1, changes in the levels of CD under the influence of the laser are determined by the buffer capacity of the irradiated postresuscitation blood (ΔBB P). The intensity of formation of SB (ΔSB Las) correlated regularly with the lactate content in postresuscitation blood (ΔLac Las) and depended on changes in the average length of the fatty acid chain during IHNL (equation 2). A more marked increase in total AOA during IHNL corresponded to a higher concentration of dissolved O_2 (equation 3).

The relationship thus obtained between the change in total AOA and dissolved O_2 connects mathematically the two hypotheses regarding mechanisms of the biological action of the laser, both through activation of antioxidant systems and through an increase in the physical solubility of oxygen.

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