Effects on Erythrocytes

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The response of Na+/K+ -ATPase of human erythrocytes to green laser light treatment.

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The objective of this study was to investigate the response of Na(+)/K(+)-ATPase of human erythrocytes to green laser irradiation. Effects of green laser light of fluences 9.5-63.3 J.cm(-2) and merocyanine 540-mediated laser light treatment were studied. Isolated erythrocyte membranes (protein concentration of 1 mg/ml) were irradiated by Nd:YAG laser (532 nm, 30 mW) and then incubated in a medium with 2 mM ATP for 30 min. Activity of ATPase was determined colorimetrically by measuring the colored reaction product of liberated inorganic phosphate and malachite green at 640 nm. Contribution of Na(+)/K(+)-ATPase to overall phosphate production was determined using ouabain. A positive effect of green laser light on Na(+)/K(+)-ATPase activity was observed. The dependence of enzymatically liberated inorganic phosphate on light fluence showed a linear correlation (R(2)=0.96, P=0.0005) for all fluences applied (9.5-63.3 J.cm(-2)). On the other hand, MC 540-mediated phototreatment caused a suppression of enzyme activity.

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Effect of low-intensity (3.75-25 J/cm2) near-infrared (810 nm) laser radiation on red blood cell ATPase activities and membrane structure.

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OBJECTIVE: The biostimulation and therapeutic effects of low-power laser radiation of different wavelengths and light doses are well known, but the exact mechanism of action

of the laser radiation with living cells is not yet understood. The aim of the present work was to investigate the effect of laser radiation (810 nm, radiant exposure 3.75-25 J/cm(2)) on the structure of protein and lipid components of red blood cell membranes and it functional properties. The role of membrane ATPases as possible targets of laser irradiation was analyzed. BACKGROUND DATA: A variety of studies both in vivo and in vitro showed significant influence of laser irradiation on cell functional state. At the same time another group of works found no detectable effects of light exposure. Some different explanations based on the light absorption by primary endogenous chromophores (mitochondrial enzymes, cytochromes, flavins, porphyrins) have been proposed to describe biological effects of laser light. It was suggested that optimization of the structural-functional organization of the erythrocyte membrane as a result of laser irradiation may be the basis for improving the cardiac function in patients under a course of laser therapy. MATERIALS AND METHODS: Human red blood cells or isolated cell membranes were irradiated with low-intensity laser light (810 nm) at different radiant exposures (3.75-25 J/cm(2)) and light powers (fluence rate; 10-400 mW) at 37 degrees C. As the parameters characterizing the structural and functional changes of cell membranes the activities of Na(+)-, K(+)-, and Mg(2+)-ATPases, tryptophan fluorescence of membrane proteins and fluorescence of pyrene incorporated into membrane lipid bilayer were used. RESULTS: It was found that near-infrared low-intensity laser radiation changes the ATPase activities of the membrane ion pumps in the dose- and fluence rate-dependent manner. At the same time no changes of such integral parameters as cell stability, membrane lipid peroxidation level, intracellular reduced glutathione or oxyhaemoglobin level were observed. At laser power of 10 mW, an increase of the ATPase activity was observed with maximal effect at 12-15 J/cm(2) of light dose (18-26% for the total ATPase activity). At laser power of 400 mW (fluence rate significantly increased), inhibition of ATPases activities mainly due to the inhibition of Na(+)-, K(+)-ATPase was observed with maximal effect at the same light dose of 12-15 J/cm(2) (18-23% for the total ATPase activity). Fractionation of the light dose significantly changed the membrane response to laser radiation. Changes in tryptophan fluorescent parameters of erythrocyte membrane proteins and the increase in lipid bilayer fluidity measured by pyrene monomer/excimer fluorescence ratio were observed. CONCLUSIONS: Near-infrared laser light radiation (810 nm) induced long-term conformational transitions of red blood cell membrane which were related to the changes in the structural states of both erythrocyte membrane proteins and lipid bilayer and which manifested themselves as changes in fluorescent parameters of erythrocyte membranes and lipid bilayer fluidity. This resulted in the modulation of membrane functional properties: changes in the activity of membrane ion pumps and, thus, changes in membrane ion flows.

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Effect of red laser light on Na+,K(+)-ATPase activity in human erythrocyte membranes sensitized with Zn-phthalocyanine.

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OBJECTIVE: The influence of laser light (670 nm) on human erythrocyte membrane Na+,K(+)-ATPase activity in the presence and absence of Zn-phthalocyanine (ZnPc) was studied. BACKGROUND DATA: The response of erythrocyte membranes to low-power laser irradiation has not been fully elucidated. In our study, we focused on the studies on photo-induced changes of Na+,K(+)-ATPase activity. The erythrocyte membrane suspensions were incubated with 2 mM of ZnPc and next irradiated with energy doses of 19.1, 38.2, 57.3, 76.4, and 95.5 J x cm(-2). MATERIALS AND METHODS: The activity of Na+,K(+)-ATPase was assayed colorimetrically at the wavelength of 820 nm and expressed in micromol of inorganic phosphate released from ATP per mg of protein. RESULTS: The measurements of Na+,K(+)-ATPase activity in erythrocyte membranes incubated with ZnPc in the dark demonstrated that all concentrations of the dye (0.5, 1, 2, and 3 microM) stimulated enzyme activity. The concentration of 2 microM caused the smallest increase of enzyme activity, so this concentration was accepted for further studies. Irradiation of erythrocyte membranes in the presence of the dye (2 microM) significantly decreased Na+,K(+)-ATPase activity. Only for energy doses of 19.1 and 38.2 J x cm(-2) was the enzyme activity comparable to the activity of the control. CONCLUSION: It was found that irradiation with all energy doses applied caused a rise of enzyme activity. In the presence of ZnPc, significant decrease of Na+,K(+)-ATPase activity was observed.

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Low-intensity near-infrared laser radiation-induced changes of acetylcholinesterase activity of human erythrocytes.

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OBJECTIVE: The aim of the present study was to investigate the transformations of red blood cells produced by low-intensity infrared laser radiation (810 nm). BACKGROUND DATA: Low-intensity (the output power of a laser device in the milliwatt range) laser radiation as a local phototherapeutic modality is characterized by its ability to induce non-thermic, nondestructive photobiological processes in cells and tissues. However, the exact theory concerning the therapeutic effects of laser biostimulation has not been developed. MATERIALS AND METHODS: The suspensions of human erythrocytes in PBS (10% hematocrit) were irradiated with near-infrared (810 nm) therapy laser at different light doses (0-20 J) and light power (fluence rate; 200 or 400 mW) at 37 degrees C. As the parameters characterizing the cell structural and functional changes membrane acetylcholinesterase (AchEase) activity, the membrane potential, the level of intracellular glutathione, the level of products of membrane lipid peroxidation, and the cell osmotic stability were measured. RESULTS: It was found that near-infrared low-intensity laser radiation produced complex biphasic dose-dependent changes of the parameters of

AchEase reaction in the dose-dependent manner: at smaller doses of radiation (6 J) the maximal reaction rate and Michaelis-Menten constant value decreased, and at higher radiation doses these parameters increased. No significant changes of erythrocyte stability, cellular redox state (reduced glutathione or lipid peroxidation product levels), or cell membrane electrochemical potential were observed. CONCLUSION: Low-intensity near-infrared laser radiation (810 mn) produced AchEase activity changes, reflecting the effect of light on the enzyme due to energy absorption. Protein molecule conformational transitions and enzyme activity modifications in cells have been suggested as laser radiation-induced events.

J Clin Laser Med Surg. 1993 Aug;11(4):185-9.

Effect of low-power He-Ne laser on deformability of stored human erythrocytes.

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This study was designed to investigate the effect of the He-Ne laser (continuous wave, lambda = 632.8 nm, 8.5 mW in power) irradiation on human erythrocyte deformability. Blood samples were obtained from hematologically normal adult donors by venipuncture. Red cells were washed and adjusted to 30% Ht with 0.9% NaCl solution (pH 7.00). Red cell solution samples were assigned to three groups. Each sample was divided into seven 3-ml working aliquots. The aliquots in Group I were irradiated for 0 (control), 1, 3, 5, 10, 15, and 30 min within 2 hr after sampling. The aliquots in Group 2 and Group 3 were stored at 5 degrees C for 24 and 36 hr, respectively, and received similar irradiations after 12 hr (in both groups), 24 hr (in Group 2), and 36 hr (in Group 3) from sampling. Red cell deformability was measured by the Nuclepore filter filtration and presented as the filter filtration rate (FFR). The deformability shown as FFR was unchanged in Group 1 (fresh cell group) from the control value, but improved significantly in Groups 2 and 3 (damaged cell groups) after the irradiation. These results suggested that the irradiation of low-powered He-Ne lasers improved cytoskeletal protein activities in damaged erythrocytes.

Artif Organs. 2000 Nov;24(11):870-3.

Low power laser protects human erythrocytes In an In vitro model of artificial heart-lung machines.

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The protective effect of the low power helium-neon (He-Ne) laser against the damage of human erythrocytes in whole blood was examined in a perfusion model using an artificial heart-lung machine. Preserved human whole blood was diluted and perfused in 2 closed circuits with a double roller pump. The laser irradiated one of the circuits (laser group), and none the other (control group). In the laser group, erythrocyte deformability and erythrocyte adenosine triphosphate (ATP) levels were significantly higher, and free hemoglobin levels were significantly lower than those in the control group. Subsequent morphological findings by means of scanning electron microscope were consistent with these results. Low power He-Ne laser protected human erythrocytes in the preserved diluted whole blood from the damage caused by experimental artificial heart-lung machines. The clinical application of low power laser treatment for extracorporeal circulation is suggested.

Klin Khir. 2000 Nov;(11):28-9.

[The first experience of application of photo-modified erythrocytes for the treatment of the chronic arterial ischemia syndrome]

[Article in Russian]

Boiko VV, Grinevich VN, Lodianaia IN.

Efficacy of application of intraarterial erythrocytes infusion, photomodified by using low-energy laser irradiation, was proved, basing on comparative analysis of treatment results of the different groups of patients with obliterating disease of the lower extremities vessels.

Experimental study of low level laser radiation effects on human blood cells.

Siposan D, Adalbert L (Bucharest, Roumania).

Fresh blood from 40 apparently healthy individuals has been irradiated with a low level HeNe laser, using EDTA anticoagulant. Doses ranged between 0-54 J/cm2. The authors watched the relative variation to the received doses of hemoreological constants - erythrocytary and leukocytary indices, as well as the variation of some erythrocytary aggreagability indices-viscocity, BSR. Following irradiation a lowering of the erythrocytary aggreagability (viscosity), BSR, and changes of some erythrocytary and leukocytary indices have been observed. The effect of low-level laser radiation on the red cell confirms the non-resonant mechanism of this bio-stimulating radiation effect by the changes in the cell membrane, in our case the blood cells, by revitalizing the red blood cell functional capacities and by several biochemical effects on the membrane level, that are to be studied thoroughly in future studies. It is concluded that the physical-

biochemical and biological effects on blood can influence the physical-chemical parameters needed for long storage of blood products as well as the quick revitalization of the erythrocytary membrane aggressed physically and biochemically, in order to perform its oxophoric function in transfusion procedures.

Vopr Kurortol Fizioter Lech Fiz Kult. 2003 Jul-Aug;(4):10-3.

[Efficacy of laser therapy in patients with ischemic heart disease]

[Article in Russian]

Vasil'ev AP, Strel'tsova NN, Senatorov IuN.

Modification of erythrocytic membrane and the trend in clinicofunctional indices were studied in 93 patients with angina of effort (FC i-IV) in the course of treatment with laser radiation (group 1) and imitation of laser radiation (group 2). In patients of group 1 the treatment resulted in stabilization of cell membrane accompanied with positive cardiodynamic changes.

:Klin Lab Diagn. 2001 Dec;(12):22-4, 33.

[The role of erythrocyte rheological determinants in the regulation of bloodflow structure]

[Article in Russian]

Katiukhin LN.

Correlations of rheological determinants of the erythrocyte viscous characteristics were studied in normal subjects and coronary patients treated traditionally and with photohemotherapy. A rigid relationship between erythrocyte deformability and aggregation was detected in the patients. Blood exposure to He-Ne laser and UV is a potent method for correcting the blood rheology. Physiological significance of the rigid relationship of erythrocyte deformability and aggregation consists in the maintenance of the structure of flowing blood, characteristic of its native status, and represents an additional mechanism of realization of the adaptive potential of blood viscosity regulation in mammals.

J Photochem Photobiol B. 2004 Mar 19;74(1):7-12.

A comparative study of 632.8 and 532 nm laser irradiation on some rheological factors in human blood in vitro.

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The effects of laser irradiation with 632.8 and 532 nm on rheological properties of blood were comparatively studied in vitro. Under the irradiation condition of 30 mW, laser

irradiation of blood samples using a spot diameter of 5 mm with each laser, showed promising results in the modulation of hemorheological properties. When blood samples from patients with abnormally high values of erythrocyte sedimentation rate (ESR) were irradiated, the values of ESR were lowered statistically by either of the 632.8 or 532 nm lasers. The laser irradiation reduced blood viscosities at different shear rates (10-110 S(-1)) for the hyper-viscosity blood samples. Laser irradiation increased the electrophoretic mobility (EPM) of erythrocytes when the values of the sample's EPM were abnormally slow. The erythrocyte deformability was enhanced by laser irradiation when the deformability of the sample from the patients was originally poor. For verifying the improvement of laser irradiation on erythrocyte deformability, the typical erythrocyte samples with poor deformability were produced by the pre-treatment of the erythrocytes with Ca(2+). The deformability of these erythrocyte samples was also improved after laser irradiation. These results suggest that membrane-bound hemoglobin (Hbm) might be the initial site of the interaction, since Hbm is the main cause of poor deformability when erythrocytes were treated with Ca(2+). In all experiments including ESR, blood viscosity, EPM and erythrocyte deformability, the 532 nm laser demonstrated more efficient effects on modulating rheological properties than 632.8 nm laser. This wavelength effect is consistent with the absorption spectrum of hemoglobin, reflecting that hemoglobin may be one of the action targets under laser irradiation