Effect of Low-Intensity Laser Radiation of the Red Spectrum on Some Properties of Erythrocytes in Wistar Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 145, No. 1, pp. 12-14, January, 2008
Original article submitted May 8, 2007

Laser radiation of different power had various effects on the properties of erythrocytes. An increase in the radiation power from 2.2 to 25 mW/cm^2 was accompanied by a decrease in the erythrocyte sedimentation rate and an increase in erythrocyte filtration index. Radiation of 50 mW/cm^2 induced abnormal erythrocyte aggregation. Increasing the time of irradiation at power intensity of 2.2 mW/cm^2 did not potentiate its effect on the blood.

Key Words: *laser radiation; erythrocyte filtration index; erythrocyte sedimentation rate; erythrocyte aggregation*

Low-intensity laser radiation of the red spectrum (LILR, power <100 mW) modifies rheological properties of the blood. This treatment produces a strong effect on erythrocyte aggregation, decreases hematocrit, blood viscosity, and erythrocyte membrane permeability for endogenous and exogenous substances, and increases hemoglobin oxygen affinity [1-3,5,7]. However, there is no general approach to the selection and description of physical parameters of irradiation in clinical practice. The effect of LILR on such parameters of the blood as deformability and viscosity of erythrocytes and physicochemical properties of hemoglobin is manifested only at a certain duration and power of radiation [1,2]. Parameters of irradiation should be evaluated to estimate the optimal therapeutic effect of LILR in circulatory diseases.

Here we studied erythrocyte sedimentation rate (ESR), erythrocyte membrane rigidity (filtration index), size of aggregates, and shape of erythrocytes after exposure to red-spectrum laser radiation of different power. We also evaluated the dependence of these parameters on the time of low-intensity laser irradiation.

MATERIALS AND METHODS

Experiments were performed on Wistar rats (n=68) weighing 220-300 g. The animals were intraperitoneally anesthetized with 1.2 mg/100 g urethane. The blood was sampled from the carotid artery after injection of anticoagulant (heparin, 50 U/100 g), placed in tubes with heparin (9:1), and centrifuged at 3000 rpm for 10 min to isolate erythrocytes from the plasma. Erythrocyte suspension with a certain value of hematocrit was prepared.

The size of aggregates and shape of erythrocytes in erythrocyte suspension in autologous plasma (hematocrit 0.5%) were estimated in a Goryaev chamber under a microscope at ×780. ESR for erythrocyte suspension in autologous plasma (hematocrit 40%) was measured routinely after 2 h. For evaluation of erythrocyte membrane rigidity, erythrocyte suspension in physiological saline with a hematocrit volume ratio of 15% was passed through

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0007-4888/08/1451-0007

TABLE 1. Dependence of Erythrocyte Filtration Index and ESR in Rat Blood on Power Intensity and Duration of Laser Irradiation

Radiation power, mW/cm^2	Time of irradiation, min	Erythrocyte filtration index			ESR, mm/2 h		
		n	control	irradiation	*n*	control	irradiation
2.2	5	20	3.41±0.62	2.25±0.27*	10	1.85±0.59	0.96±0.53
	15	17	3.76±0.72	3.67±0.77	10	1.88±0.26	2.19±0.46
	25	17	3.23±0.71	3.46±0.80	11	1.85±0.59	0.67±0.26*
25	5	14	3.70±0.37	2.25±0.43*	12	1.41±0.31	0.69±0.16*
50	5	14	3.05±0.43	2.59±0.49	10	0.97±0.32	1.00±0.15

Note. *$p \leq 0.05$, significant differences at a two-sided significance level; *n*, number of animals. Two samples (control and treatment) were obtained from each animal.

amide filters (pore diameter 5 μ). The filtration index was calculated as the ratio of filtration time for 1 ml erythrocyte suspension to filtration time for 1 ml physiological saline.

The blood of each animal was divided into 2 equal portions: control and experimental (exposed to irradiation). The test parameters were evaluated for each portion.

Study with sensitive thermocouple probe showed that exposure of the sample to a He-Ne laser at 2.2 mW/cm^2 was not accompanied by blood heating. Blood samples exposed to light-emitting diode radiation (25 or 50 mW/cm^2) were maintained in a container under temperature-controlled flow conditions to stabilize the temperature at 37°C.

Blood samples were subjected to red radiation with power intensity of 2.2 (He-Ne laser, 632.8 nm), 25, and 50 mW/cm^2 (light-emitting diode laser, 650±5 nm). All samples were irradiated for 5 min to study the dependence of erythrocyte properties on the power of laser radiation. Blood samples were irradiated at 2.2 mW/cm^2 (He-Ne laser, 632.8 nm) to evaluate the dependence of erythrocyte aggregation on the time of laser irradiation. Control samples were studied under similar experimental conditions.

RESULTS

Erythrocyte filtration index significantly decreased during irradiation with 2.2 mW/cm^2 ($p \leq 0.05$), but no significant changes in the size and shape of erythrocyte aggregates were noted (Table 1). Erythrocyte filtration index also decreased at a radiation power of 25 mW/cm^2. ESR decreased by 2 times after irradiation at a power intensity of 25 mW/cm^2 (Table 1). Light microscopy revealed no differences between the shape of erythrocytes and size of aggregates in irradiated (25 mW/cm^2) and control samples. Irradiation at power intensity of 50 mW/cm^2 had little effect on the erythrocyte filtration index and ESR (Table 1). Visual study of morphofunctional characteristics of erythrocytes showed that control sample contains small aggregates of 2-5 discocytes, while after irradiation at power intensity of 50 mW/cm^2 the cells were presented by spherocytes and echinocytes forming abnormal aggregates (side-to-side aggregation).

Studying of the dependence of rheological properties of the blood on the time of irradiation at a constant power of 2.2 mW/cm^2 yielded heterogeneous results that were difficult to be interpreted. For example, 5-min irradiation changed only erythrocyte filtration index ($p \leq 0.05$ compared to the control, Table 1); the size and shape of aggregates remained unchanged, while ESR tended to decrease under these conditions. No differences between irradiated and control samples were revealed after 15-min irradiation (Table 1). Increasing radiation exposure to 25 min was followed by a decrease in ESR (more than by 2 times, $p \leq 0.05$), but had no effect on erythrocyte membrane rigidity (filtration index did not change, Table 1), shape of erythrocytes, and size of aggregates.

It is unclear why blood parameters remain unchanged during 15-min irradiation. Moreover, the cause of variations in the effect of irradiation for 5 and 25 min remains unknown. The decrease in ESR induced by 25-min irradiation is probably related to photodynamic processes during long-lasting exposure, which results in an increase in erythrocyte z-potential. These changes contribute to deceleration of bridge aggregation and protect erythrocytes from drawing together, which determines aggregation over the 1st minutes [4,6].

Our experiments revealed a nonlinear dependence of erythrocyte aggregation and deformability on LILR power intensity and duration of exposure: the increase in the power or time of laser exposure does not necessarily potentiate its effect on the

blood. For example, increasing the time of irradiation is not equal to radiation at a higher power intensity. Irradiation of different duration and power has various effects on viscoelastic properties of the erythrocyte membrane and rheological characteristics of the whole blood.

REFERENCES

1. E. B. Zhiburt, N. B. Serebryanaya, E. N. Rozhdestvenskaya, *et al*., *Pat. Fiziol. Eksper. Ter*., No. 3, 6-7 (1998).
2. L. I. Irzhak, E. A. Zotova, and S. A. Mamaeva, *Byull. Eksp. Biol. Med*., **123**, No. 1, 15-17 (1996).
3. G. I. Klebanov, N. Yu. Shuraeva, N. G. Sidorina, *et al*., *Lazernaya Meditsina*, **8**, No. 4, 26-31 (2004).
4. V. A. Levtov, S. A. Regirer, and N. Kh. Shadrina, *Blood Rheology* [in Russian], Moscow (1982).
5. V. P. Tereshchenko, T. A. Yavorskaya, and T. A. Kolodyazhnaya, *Lazernaya Meditsina*, **9**, No. 2, 24-26 (2005).
6. A. L. Chernitskii and A. V. Vorobei, *Structure and Functions of Erythrocyte Membranes* [in Russian], Minsk (1981).
7. A. V. Shaposhnikova and T. A. Linnik, *Estestvennye Nauki*, **3**, No. 4, (1992).