

MORPHOLOGICAL AND FUNCTIONAL PROPERTIES OF ERYTHROCYTES UNDER STRESS AND EXPOSURE TO LOW-INTENSITY LASER RADIATION

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Abstract. The work aimed to study the protein spectrum, morpho-functional state of erythrocyte membranes under stress and correction by low-intensity laser radiation. The experiments were carried out *in vitro*. Low-intensity laser radiation with a wavelength of 830 nm and a power of 90 mw was used for irradiation. Laser therapy was performed using an autonomous laser shower «MarsIK» (NPO «Petrolaser», St. Petersburg). The protein fractions of red blood cells were analyzed by electrophoresis, the morphology of red blood cells by laser interference microscopy, the electrophoretic mobility of erythrocytes was measured by microelectrophoresis in our modification and the concentration of malondialdehyde in red blood cells by reaction with thiobarbituric acid spectrophotometrically. It is proved that the change of protein fractions in the erythrocyte membrane under stress, a decrease in the amount of spectrin, band 3 protein, glycophorin and ankirin. The stress is characterized by an increase in the number of echinocytes, stomatocytes and degenerative-altered red blood cells. Exposure to LLLT on blood samples determined the recovery of the studied parameters to the control group values (physiological norm). The role of the discovered metabolic changes in RBC in stress and their correction by LLLT is under discussion.

Keywords: low-intensity laser radiation, red blood cells, electrophoretic mobility of red blood cells, lipid peroxidation, membrane proteins, morphology.

List of Abbreviations

LLLT – Low-intensity laser therapy

MDA – Malondialdehyde

RBC – Red blood cells

SDS – Sodium dodecyl sulfate

Introduction

A lot of drugs having an anti-stress effect on animals are elaborated and approved by now. Those are tranquillizers, antioxidants, various vitamin-mineral additives, salt compositions, etc. However, the studies of this problem continue. They are focused on searching for new, more efficacious, accessible and cheap remedies with high adaptability to their use, which would not accumulate in the body (Himicheva & Samusenko, 2016).

In our opinion, lower-intensity laser therapy is one of such remedies. Currently, low-intensity laser therapy (LLLT) is widely used in various fields of medicine. However, the implementation of LLLT carries out predominantly empirically. Undoubtedly, the study of the

mechanism of LLLT interaction with live bodies and the enlargement of its sphere of application is a crucial task for medicine (Deryugina *et al.*, 2019; Zalesskaya & Sambor, 2005).

In connection with the above, the research purpose is to study the impact of the LLLT on the RBC electrophoretic mobility, Malondialdehyde (MDA) concentration, the spectrum of proteins of RBC membrane and RBC morphology of cattle *in vitro* experiments.

Materials and Methods

The study was conducted with the permission of the local ethics committee at the Lobachevsky State University (the protocol No. 47 of 11.09.2017).

The object of the study was the whole blood of healthy animals that had undergone technological stress (the experimental group) and the blood of non-stressed animals (the control group).

The blood of the experimented group animals had been irradiated for 15 minutes, then

the RBC electrophoretic mobility was studied, the MDA concentration in RBC, spectrum of proteins of RBC membrane and RBC morphology were defined. The blood of not irradiated animals served as a control specimen. The indices were studied in one hour after the irradiation. The irradiated and not irradiated blood had been centrifuged three times at speeds of 3000 rpm for 10 minutes.

The blood was placed in Petrie dish, diameter 3 cm at a distance of 1cm from the cell membrane surface. Autonomous laser shower MarsiK («Petrolaizer», Saint Petersburg) working in pulse regime with wave length 890 nm, was used as a laser irradiation source. 10 samples were made in each series.

The washed RBC suspension was prepared in order to assess the RBC electrophoretic mobility. The washed RBCs had been centrifuged three times in 1500 rpm with 0,9% HCl solution for 10 minutes. The cell suspension was solved in 10 mM Tris-HCl buffer; (pH 7,4). The RBC electrophoretic mobility was measured by method of microelectrophoresis by using the cytopherometer in our modification (Deryugina *et al.*, 2016), recording the time of RBC passing 100 μ m distance in Tris-HCl buffered solution, pH 7.4, at a current of 12mA. RBC electrophoretic mobility value was defined using the formula:

$$U = S/T \times H,$$

where S – distance to which the cells moved, T – time, H – gradient of electric potential. The value of potential gradient was determined according to the formula:

$$H = I / g \times \chi,$$

where I – amperage, g – chamber cross section, χ – electrical conductivity of the media conductivity of the media.

The lipid peroxidation intensity was defined by absorption spectrophotometry method based on the level of lipid peroxidation TBA-reactive substances among which malon dialdehyde (MDA) is one of the most numerous. A standard practice (Heath & Packer, 1968; Kamyshnikov, 2002) with modifications was used in order to define the MDA. This practice is based

on the formation of coloured trimethine complex with 532 nm maximum absorption when it interacts with thiobarbituric acid.

The separation of RBC membrane proteins was made with electrophoresis method with use of Sodium dodecyl sulfate (SDS). The electrophoresis was made by Laemmli method (Laemmli, 1970) with use of Mini-PROTEAN Tetra Cell apparatus (Bio-Rad, USA).

The RBC complex phasemetry was studied by laser modulation interference microscopy method with use of laser interference microscope MIM-340 (Ekaterinburg, Russia). The diode laser with 655 nm wavelength equipped with the 20 \times objective was used as the source of coherent radiation. The surface resolution is up to 15 nm, the vertical resolution is 0,1 nm (NA = 0.65). The technical characteristics of this microscope permit to study the tools which relief depth is up to 600nm. Images were captured with CCD camera VS-415U (NPK Videoscan, Russia) with resolution 782 \times 582 pixels. The morphology of native cells was studied without any preliminary fixation. It permits to visualize the modification of cells in on-line regime and to study the cell morphology.

The BIOSAT software was used for statistical processing of finding. The analysis-of-variance methods were used. Statistical significance of differences of Mean Bias if conditions of normality of distribution and dispersion equality are met was examined with use of Student's t-test.

Results

The study revealed that the blood of animals suffered a stress had the following changes. The RBC electrophoretic mobility decreased by 32%, the MDA concentration increased by 65% relative to the control group animal value (Table 1). LLLT impact on the blood of not stressed animals did not significantly alter RBC electrophoretic mobility and MDA concentration. The impact of LLLT on the blood of the stressed animals provoked the restoration of the studied indices up to physiologically normal state value (control group).

The study of protein spectrum in the blood specimen showed that the treatment with LLLT

Table 1

RBC electrophoretic mobility and MDA concentration of RBCs in the studied groups, (M ± m)

Group of animals	RBC electrophoretic mobility, (µm cm B ⁻¹ c ⁻¹)	MDA (nmol/ml)
Intact (control)	1.09 ± 0.08	2.04 ± 0.33
Intact + LLLT	1.01 ± 0.10	2.25 ± 0.31
Stress	0.75 ± 0.07*	3.37 ± 0.45*
Stress + LLLT	1.09 ± 0.11°	2.79 ± 0.43°

«*» – statistically different from “Intact” group, *p* < 0.05,

«°» – statistically different in the “Stress + LLLT” group relative to the “Stress” group, *p* < 0.05

Table 2

Spectrum of erythrocyte membrane proteins (%) of study groups, (M ± m)

Proteins	Intact (control)	Intact + LLLT	Stress	Stress + LLLT
Spectrin α	2.50 ± 0.44	2.35 ± 0.37	2.03 ± 0.65	3.19 ± 0.86°
Spectrin β	1.63 ± 0.11	0.98 ± 0.29*	1.21 ± 0.31*	2.01 ± 0.62°
Ankyrin	2.75 ± 0.15	1.72 ± 0.01*	2.05 ± 0.25*	2.21 ± 0.22
Protein p.3	23.22 ± 1.05	24.09 ± 1.13	21.75 ± 1.01*	23.83 ± 1.57
Protein p. 4.1	10.74 ± 5.56	12.17 ± 1.32	13.98 ± 4.09	9.91 ± 4.11
Glycophorin A	9.18 ± 1.05	11.04 ± 0.99*	11.91 ± 2.05	11.81 ± 1.84
Protein p.4.9	12.88 ± 4.23	10.69 ± 5.72	11.99 ± 4.22	11.09 ± 1.21
Actin	20.88 ± 0.85	16.57 ± 0.76*	21.04 ± 2.06	18.69 ± 1.34
Glycophorin C	16.22 ± 0.34	20.41 ± 1.76*	14.04 ± 0.23*	17.26 ± 0.49°

«*» – statistically different from “Intact” group, *p* < 0.05,

«°» – statistically different in the “Stress + LLLT” group relative to the “Stress” group, *p* < 0.05

Table 3

Erythrocyte morphology in the study groups (%), (M±m)

Group	Erythrocyte morphology			
	Discocytes	Echinocytes	Stomatocytes	Degeneratively altered
Intact (control)	87.26 ± 1.15	7.89 ± 0.98	3.32 ± 0.12	1.53 ± 0.22
Intact + LLLT	88.95 ± 1.64	6.89 ± 0.85	3.01 ± 0.48	1.15 ± 0.32
Stress	27.14 ± 2.32*	56.95 ± 1.12*	10.59 ± 1.00*	5.32 ± 0.87*
Stress + LLLT	69.14 ± 4.68*°	18.42 ± 2.78*°	8.19 ± 0.75*°	4.25 ± 0.66*

«*» – statistically different from “Intact” group, *p* < 0.05,

«°» – statistically different in the “Stress + LLLT” group relative to the “Stress” group, *p* < 0.05

of the intact animals provoked a real decrease in the concentration of spectrin and the increase in the number of Integral membrane protein – glycophorin (Table 2). The level of spectrin decreased by 16% whereas the level of glycophorin C increased by 35%. RBCs of the stressed animals had a lower

concentration of spectrin by 21%, ankyrin by 25%, protein band 3 by 6%, glycophorin C by 18% than that of the control animal group (*p* < 0.05). After the LLLT treatment of the experimental group cows the protein level of the RBC membranes recovered to the control group value.

The analyzes of dimensional parameters of RBC phase interference portraits of functioning RBC showed that the LLLT treatment did not provoked any considerable changes in morphological forms of the intact cells (Table 3). But the stress provoked the decrease in the discocyte number and a considerable increase in the number of echinocytes (by a factor of 7 relative to the control group value), of stomatocytes (by a factor of 3) and of degenerative-altered forms (by a factor of 4,5). After the analyzes of morphological parameters of RBCs suffered a stress and then treated with LLLT, it was established that the number of discocytes increased and it was caused by the decrease in echinocytes and stomatocytes.

Discussion

By discussing LLLT effect on RBCs it should be noted that LLLT did not affect membrane elotronegativity, lipoperoxidation processes and morphology. LLLT neutralized the negative processes provoked by the stress such as the decrease in RBC electrophoretic mobility, intensification of lipid peroxidation and the increase in pathogenic forms of RBCs. The stabilizing effect of LLLT on RBC indices in stress reaction modeling in experiment on animals were mentioned by us earlier (Deryugina *et al.*, 2019).

The electromagnetic nature of LLLT implies interaction of LLLT with multiple regulatory mechanisms in living systems. The most important regulatory system of cells is the system of free-radical processes (Dröge, 2002). Active oxygen species can directly damage vital cellular components such as lipids and proteins (Hensley *et al.*, 2000).

The change in reactive oxygen species is a secondary reaction, while the primary reaction to the action of LLLT is a change in the function of photoacceptors (Xu *et al.*, 2008). Hemoglobin and catalase molecules are LLLT photoac-

ceptors (Sutherland, 2002). A decrease in membrane-attached hemoglobin under the action of LLLT has been shown (Mi *et al.*, 2005). Catalase can determine antioxidant effects of LLLT (Kujawa *et al.*, 2014).

Also, LLLT action can be carried out through proteinkinase C (Zhang *et al.*, 2008) and phosphorylation of membrane proteins (Govekar & Zingde, 2001). Based on the results obtained we can conclude that the effect of LLLT on erythrocytes after stress is realized indirectly through acceptors and changes in lipoperoxidation processes, which may cause changes in enzyme activity. This leads to changes in the protein component of erythrocyte membranes.

The revealed protein changes probably may be the reason of increase in the deformability of RBCs which is shown in some papers studying the LLLT effect (Mi *et al.*, 2004; Alves *et al.*, 2013). In its turn, the increase in electronegativity of RBC membranes and, as consequence, a positive change in RBC microrheological properties mentioned in our paper may provoke an improvement of gas transmission function and of metabolic processes in the whole organism.

Conclusion

Having regard to the above it is possible to suppose that LLLT may drive to the correction and stimulate the processes of defense and adaptation, i.e., the mechanisms of sanogenesis of animals with disturbed functions of homeostasis as a consequence of various stress and pathogenic factor impact.

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Conflict of interest statement. Nothing declared.

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