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The Use of Low Intensity Laser Therapy for the Reduction of Technology Stress of Cows

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Abstract—**Morphological and biochemical changes in RBCs of cattle suffered a technology stress and its correction by LILT are analyzed in the paper. The study object was the whole blood of physiologically healthy black-and-white cows suffered a technology stress and the blood of not stressed animals. The blood of experimented animals was and then we studied the RBC electrophoretic mobility, the MDA concentration in RBCs, the RBC membrane protein spectrum and the RBC morphology. Negative processes provoked by stress such as the RBC electrophoretic mobility decrease, the lipid peroxidation intensification, and the increase of RBC pathological forms was stopped by LILT. After the LILT the protein number in RBC membranes of stressed animals restored to the control group value.**

Keywords— technology stress, RBCs, cattle, low intensity laser therapy (LILT), RBC electrophoretic mobility, malondialdehyde, glutathione

I. Introduction

The study of animal adaptation and stress resistance in contemporary cattle breeding is of current importance now because animal stress becomes more frequent. It's a result of animal welfare change, in-plant noise, rearrangements, and other technologic manipulations [1].

It's proved that the stress influence on productive animal organism decreases the resilience of the animal organism [2, 3]. So the prevention or the decrease of negative stress consequences is on the most important factors of health maintenance, of animal productivity increase and of the production cost decrease.

Now the research, study, and application of new technological methods which could inhibit the consequences of technology stress are of current importance.

The low intensity laser therapy (LILT) may serve a remedy. The laser radiation influence offers a real opportunity to decrease technology stress, to regulate the productivity and the production quality. It's a common knowledge that LILT stimulating activity provokes the activation of organism systems, it accelerates metabolism, it mobilizes animal immunodefences and it makes influences on proteosynthesis. That's why the study of technology stress correction by LILT has scientific and practical value [4].

It's possible to study the influence of stress on the organism and its correction by making biochemical and physiological fundamental research of cell reaction to stress.

The hemic system is a trustworthy clinical score which permits to assess the organism state. RBC membranes are a good model for studying the state of body membranes. That's why it's necessary to discover functional morphological and biochemical shifts in RBC under the influence of stress and LILT. Such analyze is important in theoretical as well as in practical aspects. [5, 6].

II. Materials and Methods

The study object was the whole blood of physiologically healthy black-and-white cows suffered a technology stress (experimental group) and the blood of not stressed animals (control group). The groups (control and experimental) were formed on the principle of groups analogs by sex, age, average body weight, phenotypic characteristics. There were 10 animals in each group.

The blood of both groups was irradiated for 15 minutes and then we studied the RBC electrophoretic mobility, the MDA concentration in RBC, the RBC membrane protein spectrum and the RBC morphology. The control parameter was the not irradiated blood of the same animals. The study was made in one hour after the beginning of the radiation.

The therapeutic laser apparatus “Uspekh” (“Voskhod”, Russia) was used as a source of irradiation. It’s a compact autonomous matrix consisting of 10 laser diodes. It works on pulse frequency 415 Hz at,890 nm wavelength. The total peak-pulse power of the radiation is 30W. The minimum value of average power density in the plane of the output window was 193 μW/cm2. The surface of the outlet is 20 cm2. 10 tests were made in each series.

The RBC electrophoretic mobility was measured by microelectrophoresis method in our modification [7]. The MDA concentration was measured basing on the formation of colored trimethine complex. The maximum absorption was achieved when the wavelength was 532 nm. The proteins were separated by SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) with use of vertical electrophoresis chamber VE-10 (Helikon). The lipids were extracted by Folch method with use of the system: chloroform, methanol, water.

The RBCs of different forms were identified by their image in transmitted light. The volume change and phase height distribution was studied by laser interference method with laser interference microscope MIM-340 (Ekaterinburg, Russia) equipped with the 30× objective (NA=0.65) and laser at 650nm. 5μl of the solution with cells were put on the mirror object surface. So we registered the RBC interference images. Images were captured with CCD camera VS-415U (NPK Videoscan, Russia) with resolution782×582 pixels. The phase image reconstruction from the whole interference image was done by phase step method.

Programs Microsoft Excel and BIOSTAT were used for statistical processing of finding. Student's t-test was used for comparison of two groups.

III. Results and discussion

The study of RBC morphology defining the RBC possibility to provide the organism by oxygen showed that the control group animals had three basic kinds of erythrocytes such as biconcave RBCs, echinocytes, stomatocytes. The RBC three-dimensional shape could be described by its sphericity coefficient k. It may be defined by the RBC thickness index in the center to that in radius center ratio. The sphericity coefficient was determined for each RBC specially during interferogram computer processing. Biconcave form is a normal form. The sphericity coefficient of such RBCs is lower than one and their functional abilities are maximal. Two other kinds of RBC forms are pathological. Their sphericity coefficient is higher than one. The stress provoked following changes [8]. The number of discocytes decreased whereas the number of echinocytes, stomatocytes and degenerative changed forms increased considerably. So they are higher than that of control group animal value 7, 3, and 4,5 times respectively. The RBC morphology study showed that the LILT did not provoke any considerable change in cell morphology and the RBC sphericity coefficient was lower than one. However, the treatment of stressed animals by LILT provoked the increase of discocyte number because of echinocyte number decrease.

The structural state of RBC membranes determines their ability to sorb various substances. The change of RBC sorption capacity may provoke the toxin accumulation in plasma or the disturbance of membrane permeability for other metabolites.

It was established by the experiment that the sorption capacity of RBC membranes decreased after the stress. It makes evident that the cellular membrane is pathologically overcharged and that it’s not able to transport metabolites in the blood stream. The sorption capacity of RBC membranes increased after the influence of LILT on intact animal blood.

The change of cell morphology is probably reasoned by the increase of RBC membrane electronegativity and with the intensification of lipid peroxidation. It’s proven by the findings (Table 1).

Table 1. RBC electrophoretic mobility and MDA concentration in experimented animal groups (M±m)

Animal groups	RBC electrophoretic mobility (μm·cm/V^{-1}·c^{-1})	MDA, (nmol/ml)
Intacts (control)	1.09±0.08	2.04±0.33
Intacts after the LILT treatment	1.40±0.10*	1.25±0.31*
Stressed	0.75±0.07*	3.37±0.45*
Stressed after the LILT treatment	1.09±0.11°	2.79±0.43°

Notice: «*» - statistically different from “Intact” group, p< 0.05;
«°» - statistically different in “Stress+ LILT” group from “Stress” group, p<0,05.

The RBC electrophoretic mobility of suffered a technology stress animals was by 32% lower than that of control animals and their MDA concentration was by 65% higher. The LILT treatment of not stressed animals provoked the increase of RBC electrophoretic mobility by 28%. On the contrary, the MDA concentration was by 39% lower than that of not treated animals. The LILT influence on stressed animal blood provoked the restoration of researched indices to physiologically normal state value (control group).

The study of Heinz's bodies which are markers of influence toxicity showed that their number increased considerably in technological stress. The stressed animals treated by LILT had less pathology in RBC than those which did not receive such treatment. The influence of LILT on intact organism did not provoke any increase of Heinz's bodies number during all the experiment (Table 2).

Table 2. The number of Heinz's bodies in blood films of intact and stressed animals

Animal groups	The number of Heinz's bodies (per 1000 RBC)
Intacts (control)	2±0,81
Intacts after the LILT treatment	1,25±0,25
Stressed	20,50±3,22*
Stressed after the LILT treatment	12,50±2,32*

Notice: * statistically different from intact group, p<0.05

It should be noted by discussing the LILT effect on animal RBC that after the irradiation of blood specimen the RBC membrane electronegativity increased, lipid peroxidation processes became less intensive and at that time the cell morphological form preserves [9, 10]. Negative processes provoked by stress such as the RBC electrophoretic mobility decrease, the lipid peroxidation intensification, and the increase of RBC pathological forms was stopped by LILT.

It was established that the shape of RBCs defines not only their kinetic performance i.e. the ability to circulate in blood especially in microvessels but also to the ability to realize basic biochemical processes which reason the functional specificity of RBCs. One of important RBC particularities is the participation of RBCs in glycolysis during which the ATP, their base energy source, appears. The poikilocytosis i.e. the change of RBC shape may be a consequence of cell cytoskeleton disturbance [11, 12, 13].

The study of protein spectrum in blood specimen discovered that after the LILT treatment of intact animals the quantity of spectrinaktine complex proteins decreased significantly and the quantity of integral proteins increased (Table 3, 4).

TABLE 3. CHARACTERISTIC OF RBC MEMBRANE PROTEINS FOR STUDIED GROUPS OF ANIMALS (%, (M±M)

Indices	Animal group	
	Intact (control)	Intact after the LILT treatment
Spectrin α	2.50± 0.44	2.35 ±0.37
Spectrin β	1.63 ±0.11	0.98 ±0.29*
Ankyrin	2.75± 0.15	1.72 ±0.01*
Band 3 protein	23.22± 1.05	24.09 ±1.13
Band 4.1 protein	10.74 ±5.56	12.17 ±1.32
Glycophorin A	9.18±1.05	11.04 ±0.99*
Band 4.9 protein	12.88± 4.23	10.69± 5.72
Actin	20.88 ±0.85	16.57 ±0.76*
Glycophorinн C	16.22±0.34	20.41 ±1.76*

Notice: «*» - statistically different from "Intact" group, p< 0.05;
«°» - statistically different in "stress+ LILT" group from "Stresst" group, p< 0.05.

TABLE 4. CHARACTERISTIC OF RBC MEMBRANE PROTEINS FOR STUDIED GROUPS OF ANIMALS (%,(M±M)

Indices	Animal group	
	Stressed	after the LILT treatment
Spectrin α	2,03± 0,65	3,19± 0,86°
Spectrin β	1,21 ±0,31*	2,01± 0,62°
Ankyrin	2,05± 0,25*	2,21± 0,22
Band 3 protein	21,75±1,01*	23,83± 1,57
Band 4.1 protein	13,98 ±4,09	9,91± 4,11
Glycophorin A	11,91± 2,05	11,81± 1,84
Band 4.9 protein	11,99± 4,22	11,09± 1,21
Actin	21,04 ±2,06	18,69± 1,34
Glycophorinн C	14,04± 0,23*	17,26 ±0,49°

Notice: «*» - statistically different from "Intact" group, p< 0.05;
«°» - statistically different in "stress+ LILT" group from "Stresst" group, p< 0.05

The quantity of spectrin, actin, and ankyrin decreased by 40%, 21%, and 38% respectively whereas the glycophorin A and C quantity increased by 20% and 25% respectively. As for stressed animals, the concentration of spectrin decreased by 26%, ankyrin –by 25%, band 3 protein – by 7%, glycophorin C – by 15% relative to control animal group RBC (p<0,05) .

After the LILT treatment, the quantity of RBC membrane proteins in experimented cow group restored to the value of the control group [14, 15, 16].

The morphometric study of RBC surface relief discovered that the intact group animals had a typical form of discocytes. The RBC membrane had a plane surface. The intracellular structures were well-distributed. So, hemoglobin and therefore refraction index were well-distributed. After the LILT treatment, the RBC phase image preserved the form of discocytes but it had not a smooth doughnut shape but it was so called rough with outgrowths and venters. Such transformation of RBC form in a smooth provoked by the LILT is probably associated with the changes in cytoskeleton structure and with the redistribution of hemoglobin in the cytoplasm and in submembrane areas. It should be highlighted that though the discoid form and the size of RBCs are similar in the control group and in the LILT group, a significant difference in phase images in these two groups is evident. It was revealed by phase interference microscopy.

The phase portraits of the stressed group animals had the following changes. The echinocytes appeared, the architectonics and the appendices number changed, the cell sphericity increased. There were microvesicles on the surface of some echinocytes. The absorbency of cytoplasm changed and it makes evident that the hemoglobin in RBC cytoplasm was not more well-distributed. The geometrical dimension of cells changed: the volume of the most cells increased but the volume of some cells decreased. The number of degenerative changed forms (like a deflated ball) increased progressively. After the treatment of the stressed animals by LILT, the number of cells with appendices decreased. The discocytes had different degree of biconcavity. It manifested in different flection and deepness of physiological escovation.

The change of optical density made evident that the density of packaging of hemoglobin molecules increased. At that the inconvertible changed (prehemolytic) RBCs such as stomatocytes and degenerative changed cells conserved.

The LILT provokes the changes of not only protein spectrum but also of lipid profile of membranes. But these changes aren't pathological and they provoke the activation of physiological processes.

The analyses of RBC membrane phospholipid spectrum treated by LILT makes evident that there are not any changes which could mark that the stress took place. In stress the level of lysophospholipids increased, the level of phosphatidylcholines decreased. It means that the phospholipase A2 activates. The activation of phospholipase A2 provokes the inhibition of Sphingomyelin phosphodiesterase activity and the decrease of sphingomyelin level. The treatment of the stressed animals by LILT provoked the increase of sphingomyelin and phosphatidylcholine level. The decrease of phosphatidylcholine/sphingomyelin coefficient decreased the fluid properties and increased the microviscosity of lipid bilayer.

So, the researches confirmed that the morphology of RBC changes in stress and it reflects the state of organism in the whole. The electromagnetic nature of low intensity laser radiation presumes that it may interact with various regulatory mechanisms in living systems. The LILT modulates various biological processes without damaging hydrogen binding in tissues but it provokes the photochemical effect. There is a very important regulatory system of cells. That is the system of free-radical processes with which various biological phenomenons including the change in morphology are associated. In addition, the activation of antioxidant defense is revealed. For its turn, the antioxidant effect of LILT probably influences the structure of RBC membranes which determines their surface potential. The dissociation of main and acid groups of the membrane surface creates something like a mosaic composed from positive and negative charges. As the acid groups predominate the RBC has an excess negative charge in its surface. It should be noted that though the RBC electrophoretic mobility increases under the influence of LILT the discoid form of erythrocytes persists. Probably, it is associated with charge redistribution in glycocalyx because the type of interaction between integral and peripheral proteins changes. The cytoskeletal proteins form a connection with integral proteins i.e. dimers of α and β spectrins, actin, band 4 proteins are associated with band 3 integral proteins and glycophorin. It is a common knowledge that the spectrin accomplishes a structural function. But besides it is responsible for membrane permeability, it makes influence on properties of ATPases which are connected by structure with spectrin-actin complex [17].

In stress the modification of spectrin and the disturbance of lateral mobility of integral proteins take place [18]. It was manifested during the experiment by the decrease of β spectrin, by Na+/K+-ATPase inhibition and consequently accumulation of calcium in the cytosol of erythrocyte. When the concentration of Ca2+ in cytosol decreases from 6 μm up to 50 μm the viscosity and fragility of RBCs decrease too [19]. At the same time the ankyrin displaces from protein band 3. It makes the interaction membranes - cytoskeleton weaker [20]. Modified forms of RBCs appear. The echinocyte deformation disturbs a normal ordering of cell deformation [21]. This disturbance is associated with the decrease in actin and spectrin quantity [22]. For its turn, mechanical deformation is the main stimulus for the release of ATP from RBCs. It may conduce the vasodilation of vessel and it regulates local blood flow [23, 24].

Our findings showed that LILT may make a remedial action and stimulate the processes of defense and adaptation i.e. the sanogenesis of animals with a disturbance in homeostasis which are consequences of pathologies and stress.

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