ORIGINAL ARTICLE

Morphofunctional parameters of erythrocytes in blood of chickens at adaptation to different light status

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Abstract

Aim: The aim is to study the dynamics of morphofunctional parameters of erythrocytes in the process of chickens adapting to the changes in light status. **Materials and Methods:** Atomic force microscopy was used in the study to find out changes that were primarily related to the ability of cells to deform in various light conditions. **Result and Discussion:** This study revealed a reduction in the size of erythrocytes due to the depth and width of the paranuclear area, as well as a fall in the stiffness of cell membranes that indicate changes in contact and elastic properties of cellular structures, which provide oxygen transport by the blood and microcirculation.

Key words: Adaptation, chickens, depth of paranuclear area, erythrocytes, light status, modulus of elasticity, stress, surface area, surface potential, width

INTRODUCTION

Experimental and clinical studies have revealed that changes in the morphometric properties of cells are among the universal components of adaptive and compensatory reactions of the organism with dysfunctions of various genesis and severity. It gives an objective assessment of the physiological state of the organism and predicts the development of an adaptation process in changing environmental conditions.[1-4]

The parameters of the erythrocyte geometry characterize the properties of red cells to deform under external impact and to change the viscous properties of blood, which largely determines the quality of capillary blood flow and indicates a certain change in the rheological properties of the blood.[1,5-7] In this regard, the study of structural and metabolic status of red blood cells of chickens when adapting to production factors is not only of scientific but also of practical interest.

Atomic force microscopy (AFM) makes it possible to study the morphometric and functional properties of not only the cell as a whole but also its individual parts. On the AFM data, it is possible to diagnose the state of the cell and find out pathology when creating the physical and mechanical image of the cell, which includes a set of parameters characterizing the properties of the cell membrane. The awareness of physical and mechanical properties of erythrocytes is necessary for early observation of changes in cell functions and predictions of possible disorders in the circulatory system.[8]

The aim of the study was to reveal the dynamics of some morphometric and functional parameters of the erythrocytes in the blood of chickens in the process of adaptation to different light status.

MATERIALS AND METHODS

The experimental part of the study was carried out on the laying chicken of the cross "Haysex Brown" of 10 months in

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3 groups, 16 animal units each in the vivarium. The 1st group of chickens, under control, was kept under light status for 12 h, that is, 12 h light (L) - 12 h darkness (D); the chickens of the second group - under light status 7 h L - an hour D; the chickens of the 3rd group - under the light status 2 h L - 3 h D - 5 h L - an hour D - 4 h L - 9 h D. The adaptive characteristics of the chicken organism have been studied for 30 days.[9]

To study the morphofunctional parameters of the cells, 5 ml of chicken blood was taken from the axillary vein on the 6th, 16th, and 30th days of adaptation. Cells scanning ($n = 10$) were carried out on the INTEGRA Vita (NT MDT, Zelenograd) AFM in the research laboratory "Physiology of Adaptation Processes" of Belgorod State University in a semi-contact method. The results were processed using software Nova 1.0.26 Build 1397 (NT MDT). Potential of the cell surface was evaluated by the Kelvin probe. The erythrocyte deformability index was calculated with the formula: ID = S/V, where S is the erythrocyte surface area, V is the volume. The reliability of the differences was assessed according to Student's t-criterion.

RESULTS AND DISCUSSION

The analysis of morphometric parameters of erythrocytes allows studying cellular mechanisms of adaptive and compensatory reactions of the organism. Table 1 presents the study results of geometric parameters of erythrocytes in the blood of chickens in the process of adaptation to different light status.

On the 6th day of adaptation to different lighting status in geometric parameters of red blood cells of chickens was found a decrease in surface area by 8.4% and 22.5% in groups 2 and 3, respectively, the volume and width of cells in group 3 was 35.3% and 12.1%. At the same time, an increase in width of the paranuclear area of erythrocytes in the blood of chickens in group 2 and a decrease in this parameter were found in group 3. The depth of the paranuclear space of erythrocytes in chickens blood in experimental groups significantly increased by 254% and 112%, respectively, in comparison with those in the control group.

On the 16th day of adaptation, the following significant differences were traced in the geometric parameters of

Table 1: Dynamics of geometric parameters of erythrocytes in chickens blood when adapting to different lighting status

Geometric parameters	Days	Groups		
		1	2	3
Surface area, μm²	6-e	90.8±2.1	83.2±2.5**	70.4±3.5**
	16-e	91.4±1.4	92.7±4.5	100.6±6.1
	30-e	92.2±3.2	101.1±5.1	92.5±6.3
Volume, μm³	6-e	64.1±1.8	57.9±3.1	41.5±2.1**
	16-e	65.2±1.4	55.7±2.1**	67.2±3.3
	30-e	66.8±1.7	67.7±2.5	76.6±7.8
Length, μm	6-e	10.4±0.1	9.9±0.2	9.4±0.2
	16-e	10.2±0.3	9.6±0.2*	9.5±0.4*
	30-e	10.4±0.1	9.6±0.3*	9.1±0.7
Width, μm	6-e	6.6±0.1	6.5±0.3	5.8±0.1*
	16-e	6.9±0.9	6.2±0.3	6.6±0.3
	30-e	6.8±0.4	0.4±0.1**	5.3±0.3*
Height, μm	6-e	0.9±0.02	0.9±0.1	0.8±0.03*
	16-e	0.8±0.1	1.01±0.1	0.9±0.01
	30-e	0.9±0.02	0.8±0.02*	0.9±0.1
Width of paranuclear area, nm	6-e	6.9±1.5	41.7±0.2***	2.1±0.5**
	16-e	6.2±1.2	6.0±1.2	16.5±3.5*
	30-e	7.1±0.8	6.0±1.3	2.7±1.0*
Depth of paranuclear area, nm	6-e	44.8±2.5	159.0±2.9**	94.8±2.1***
	16-e	42.3±1.6	47.0±5.1	2.3±0.4***
	30-e	48.2±2.2	25.5±3.1**	41.4±4.1

Remarks: The reliability of the differences in comparison with the data of the 1st group for $P<0.05$, **for $P<0.01$

red blood cells in the experimental group. The volume of erythrocytes in chickens blood in group 2 decreased by 14.6%; the length of erythrocytes in groups 2 and 3 - on average by 7%, the width of erythrocytes in chickens blood in group 3 by 7%. Significant changes took place in the parameters of the paranuclear space. The width of this space in group 3 increased by 158%, the depth in the same group decreased by 96%.

On the 30th day of adaptation, significant differences were found in the chickens blood in the experimental groups according to the following parameters: Length, width, height, and depth of the paranuclear space. At the same time, these morphometric characteristics of erythrocytes were lower in experimental groups than in control ones.

It should be noted that during the test there was a general tendency to a decrease in the size of cells with the restructuring of intracellular space. These changes seem to reflect adaptive changes aimed at improving capillary circulation.

The volume of erythrocyte is a value that determines the ability to provide transport function. On average, the erythrocyte volume is 60% of the maximum possible for a given surface area. This is what determines its ability to deform, which is necessary for passing through the smallest capillaries. The main function of the erythrocyte - the transport of oxygen - makes quite high demands on the possibility of deformation. To pass into the capillary, the erythrocyte is to change its shape significantly. Therefore, the speed of movement of the erythrocyte in the capillary strongly depends on its viscoelastic characteristics. If the volume of erythrocyte is large, then there are not enough surfaces for it to change its shape and pass into the microcirculatory pathway.[10-12]

The studies of recent years have shown that in the process of adaptation, primarily, the properties of erythrocytes have the ability to deform.[10,12-14] Proceeding from this, we studied the possibility of changing the shape of red blood cells in the process of adaptation to different light status. Table 2 shows the dynamics of deformability index of erythrocytes in the blood of chickens under adaption to light status [Figure 1].

The deformability of erythrocytes depends on the volume area. The larger the ratio of the area to its volume is, the more its deformable properties are. The fall in S/V ratios increases the volume of the red blood cell with excess water intake and Na. As a result, it acquires a spherical shape and becomes less deformed. The viscoelastic properties of erythrocytes favor the action of membrane permeability which leads to a rapid destruction of the erythrocyte. Thus, the ability to deform is the limiting factor of the lifespan of the erythrocyte. In general, an increase in the S/V index of erythrocytes indicates positive changes in structural and functional properties of the erythrocyte membrane.

In our studies, in group 3, on the 6th day of adaptation, the erythrocyte deformability index was significantly higher than the control indices by 21%. The research by Lutsenko *et al.* 2013, shows that the violation of deformability of erythrocytes leads to the disruption of gas exchange in tissues since erythrocytes with broken deformity fail to penetrate into narrow capsules of the peripheral circulatory system, and therefore tissue hypoxia is developed.[12]

In our studies, in group 3, an adaptation to light status caused an increase in the number of cells with much deformability. It can be recommended as an adaptation process that aims at increasing the delivery of oxygen to the microcirculation pathway.

The properties of the membrane, closely related to its surface, determine the functional characteristics of red blood cells. Table 2 presents the studies of some functional parameters of the erythrocyte membrane under chickens' adaptation to light status.

The elastic modulus of the erythrocyte membrane characterizes its average stiffness. In our studies, on the 6th day of adaptation, the indicator in experimental groups was significantly lower than those in control group on average by 65-79%. According to Tukin and Fedorova, the growth of the cell surface area is possible due to a more complete use of the membrane store.[4,9] Apparently, the cell content is distributed evenly, the elasticity of the membrane decreases,

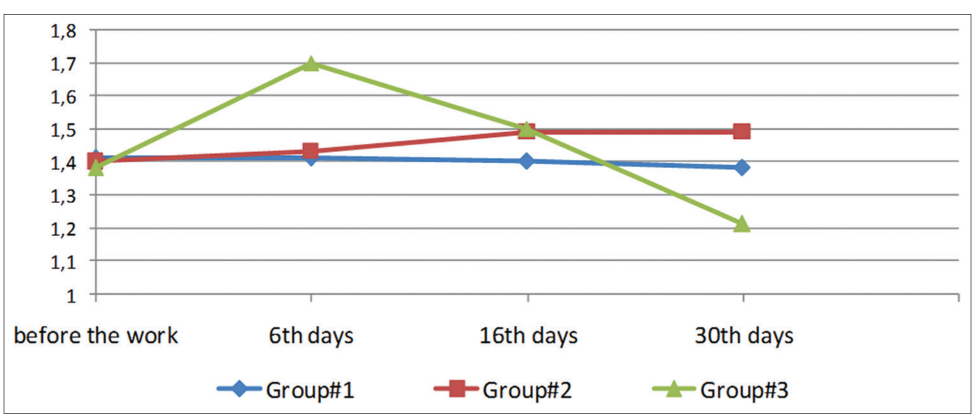

Figure 1: The index of deformability of erythrocytes in the blood of chickens when adapting to different light status

Table 2: Functional properties of the chicken erythrocyte membrane when adapting to different light status

Cell parameters	Days	Groups		
		1	2	3
Modulus of elasticity, mPa	6th	6.8±1.5	1.4±0.3**	2.4±0.01**
	16th	6.1±1.9	7.8±1.1	2.9±0.03*
	30th	5.8±2.1	3.5±0.3*	3.9±0.1
Surface potential, mV	6th	−7.6±1.4	−10.4±1.9	−5.5±1.4
	16th	−8.2±2.1	−7.4±2.6	−5.0±1.7
	30th	−7.2±1.2	−3.5±0.7*	−5.7±0.9

which increases blood flow in microvessels. This can be confirmed by the studies of Xiong and Tukin which revealed that in case the stiffness of cell membranes increases, blood flow in microvessels is bothered.[4,5,7] In the studies of Zuk *et al.*, it was also found out that an increase in the erythrocyte elastic modulus worsens the ability of a cell to bind oxygen and transport it through capillaries.[1,7,15]

It is known that blood cells suspended in plasma or physiological salt solution (erythrocytes, platelets and leukocytes) carry a negative charge at their physiological pH, which ensures their normal functioning.[10,16] In our studies, the value of the surface potential of the erythrocyte membrane on the 30th day of adaptation in the blood of chickens in group 2 was 51% higher than the values in the control group. In the studies of Neu and Meiselman, the essential role of the negative charge of the membrane is shown to prevent spontaneous aggregation of cells. Loss of charge in the erythrocyte membrane leads to a more dense relationship between them in the solution, and then to aggregation, so that the negative charge of the membrane is essential to prevent spontaneous aggregation of cells.[17] On the other hand, the charge of the erythrocyte membrane serves a purpose in circulating the blood. The repulsive forces between red blood cells and the cell wall, due to the negative charge of erythrocyte membranes and intima are important for the aggregation resistance of erythrocytes in the bloodstream.

Outputs

The studies revealed that the change in the light status causes adaptive processes that affect the shape and properties of red blood cells of chickens. During the adaptive processes in the nuclear erythrocytes of chickens, there is a tendency to a decrease in the size of the cells with the restructuring of the intracellular space. At the same time, there were observed changes in the ability of erythrocytes to deform. Functional properties of cell membranes were characterized by a decrease in average stiffness, as evidenced by a steady decrease in the modulus of elasticity. All this indicates that when the organism of chickens tries to adapt to different light status, the contact and elastic properties of the cellular structures of red blood cells change, which stimulates the transport function of erythrocytes and the circulation of blood.

CONCLUSION

1. When adapting to light status, significant changes occur in the morphofunctional parameters in the red blood cells of chickens.
2. In the process of chickens adapting to light status, the geometric dimensions of cells reduces, the volume and stiffness of cytoskeleton structures falls.
3. Cell mechanisms of body adaptation affect the depth and width of the paranuclear area of erythrocytes. The changes in the morphometric characteristics of red blood cells are associated with their ability to deform and apparently are aimed to oxygen transport function of the blood optimization and the improving of blood flow in microvessels.

REFERENCES

1. Berzosa C, Gomez-Trullen EM, Piedrafita E. Erythrocytemembrane fluidity and indices of plasmatic oxidative damage after acute physical exercise in humans. Eur J Appl Physiol 2011;111:1127-33.
2. Renaudeau D, Collin A, Yahav S, de Basilio V, Gourdine JL, Collier RJ. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. Animal 2012;6:707-28.
3. Tochilo SA, Lipnitsky AL, Akulich NV, Marochkov AV. Structural and functional changes in red blood cells during anesthesia with different anesthetics in abdominal surgery. Herald anesthesiology and resuscitation. Russ 2012;6:12-8.
4. Tukin VN, Fedorova MZ. Age-dependent changes in biochemical indices of blood and their relationship with the stiffness of membranes of hemocytes in the blood of healthy men and women. Belgorod state university scientific bulletin. Ser Nat Sci Russ 2012;3:155-60.
5. Lei J, Karniadakis GE. Probing vasoocclusion phenomena in sickle cell anemia via mesoscopic simulations. Proceedings of the national academy of sciences of the

United States of America. USA 2013;110:11326-30.
6. Moradi AR, Daneshpanah M. Detection of calcium-induced morphological changes of living cells using optical traps. IEEE Photonics J 2010;2:775-83.
7. Xiong W, Zhang J. Two-dimensional lattice Boltzmann study of red blood cell motion through microvascular bifurcation: Cell deformability and suspending viscosity effects. Biomech Model Mechanobiol 2012;11:575-83.
8. Starostová Z, Kubicka L, Konarzewski M, Kozlowski J, Kratochvíl L. Cell size but not genome size affects scaling of metabolic rate in eyelid geckos. Am Nat 2009;174:E100-5.
9. Belyaeva EY, Buslovskaya LK, Kovtunenko AY. Morphological and functional parameters of the chickens blood cells during the adaptation to the modes of lighting. Belgorod state university scientific bulletin. Ser Nat Sci Russ 2013;10:107-2.
10. Goroncharovskaya IV, Dzgoeva A, Tsarkova TG. Electrochemical interaction of red blood cells with polarized platinum electrode. Success Chem Chem Technol Russ 2015;3:32-4.
11. Kostelecka-Myrcha A. The ratio of amount of haemoglobin to total surface area of erythrocytes in birds in relation to body mass, age of nestlings, and season of the year. Physiol Zool 1997;70:278-2.
12. Lutsenko MT, Andrievskaja IA, Ishutina NA. Morphology and functions of erythrocyte membranes in pregnant women after exacerbation of herpesvirus infection. Bull Exp Biol Med J Russ 2013;1:112-4.
13. Lyssov AE, Pavlova TV. Morphologic peculiarities of red blood cells in children with acute appendicitis. Fundam Res Russ 2013;9:70-3.
14. Sokolova IA. Erythrocyte aggregation. Regional circulation and microcirculation. J Russ 2010;4:4-26.
15. Zuk A, Targosz-Korecka M, Szymonski M. Effect of selected drugs used in asthma treatment on morphology and elastic properties of red blood cells. Int J Nanomed 2011;6:249-57.
16. Bondar OV, Saifullina DV, Shakhmaeva II, Mavlyutova II, Abdullin TI. Monitoring of the zeta potential of human cells upon reduction in their viability and interaction with polymers. Acta Nat 2012;4:78-1.
17. Neu B, Meiselman HJ. Depletion-mediated red blood cell aggregation in polymer solutions. Biophys J 2002;83:2482-90.

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