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Segmented Blood Neutrophil Cytometry in Chickens When Adapting to Photojetlag

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Abstract: We analyzed the morpho-functional state (geometric parameters, parameters of the surface protrusions, the membrane elastic modulus) of chicken blood neutrophils under photojetlag by using the method of atomic force microscopy. We found that chronic stress leads to the reduction of the surface area of the neutrophils membrane and its rigidity as well as increases the width of surface protrusions with decreasing its height. These changes may indicate the decrease of the secretory activity of neutrophils and activation of their motor ability.

Key words: Adaptation, stress, neutrophils, photojetlag, cytometry, atomic force microscopy

INTRODUCTION

Neutrophilic leukocytes are the first link in the body's defense against infection caused by bacteria, fungi, viruses as well as by transformed and degenerated cells. Currently, one consider the neutrophils as primary cells that play a key role in anti-infective body defense not only because of their ability to absorb pathogens but also to release a range of microbicidal components. There is a great collection of both experimental and clinical material that allows us to consider the neutrophils activation as an emergency response determining the body's resistance. Studying both morphological and functional parameters of blood cells gives full information about their role in maintaining the homeostasis (Vasilenko *et al*., 2008; Galkin and Demidova, 2011; Gainitdinova *et al*., 2015).

Objective of this study is to study the dynamics of several morphometric and functional parameters of chicken blood neutrophils during their adaptation to chronic stress caused by photojetlag.

MATERIALS AND METHODS

We carried out the experimental art of he work in a vivarium on the 18 months chickens of hay sex brown cross formed on the analogue principle in the control group 1 and experimental group 2, each of 16 animals. The chickens were kept in cages, under cage density and feeding and watering space complying with zootechnical standards and growing technology of this cross chronic stress-photojetlag was achieved by exposing the chickens to permanent light for 3 days (Buslovskaya *et al*., 2013). To study morpho-functional cell parameters we drew the blood samples from the chicken axillary vein per 5 mL on 6, 16 and 30 adaptation day. We scanned the cells (n = 10) with an Atomic Force Microscope (AFM) «INTEGRA Vita» (NT MDT, Zelenograd) in a research laboratory "physiology of adaptation processes", SRU BelGU, by Semi-Contact Method and processed the obtained results using Nova 1.0.26 Build 1397 (NT MDT) Software. The potential of cell surface was assessed by Kelvin Probe. The differences significance was assessed by student t-test.

RESULTS AND DISCUSSION

As we found in our research, the photojetlag causes in chickens the signs of chronic stress which is evidenced by the results of the assessment of body functional state, hematological and biochemical parameters and analysis of leukogram and leukocyte indices (Buslovskaya *et al*., 2013). According to modern concepts, the characteristics of body adaptive reactions cannot be complete without an analysis of morpho-functional dynamics of blood cells;therefore, we studied both geometric parameters and functional properties of neutrophils by using the method of Atomic Force Microscopy.

Neutrophil granulocytes represent phagocytes and have a number of unique properties, namely high mobility, the ability to move easily in the tissues and produce bactericidal and cytotoxic products. Vasilenko *et al*. (2008) treat them as a peculiar "emergency response" team activated in the non-specific body defense reactions. Table 1 shows the results of a study of some geometric parameters of neutrophils.

During the experiment, the cell surface area reduced significantly, thus, it was 32.2% on day 16 and 37.9% on day 30 as compared with the control group of chickens.

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Table 1: The dynamics of the geometric parameters of blood neutrophils of chickens when adapting to photojetlag

		Cell parameters			
Groups	Days	Surface area (μm^2)	Volume (μm^2)	Height (μm)	Diameter (μm)
1	6	176.1±27.2	65.3±10.3	0.6±0.1	10.9±0.3
	16	174.8±3.8	66.2±4.2	0.6±0.1	10.6±0.4
	30	172.8±8.2	65.8±5.2	0.6±0.2	10.8±0.5
2	6	200.6±11.4	125.9±10.3**	0.4±0.1	12.6±1.4
	16	119.4±2.2**	65.7±5.9	0.5±0.6	10.6±0.4
	30	109.3±9.3**	79.5±7.1	0.7±0.1	11.1±3.1

Significance of differences compared to the data of the group 1; *p<0.05; **p<0.01

Neutrophil volume increased significantly by 92.8% on day 6. No significant changes in cell height and diameter were detected.

Gaspar *et al*. (2015) and Gainitdinova *et al*. (2015) found that the geometric parameters of neutrophils such as diameter, surface area, height and volume are changed significantly during inflammatory processes and indicate the degree of activation of polymorphonuclear leukocytes. On the other hand, the values of both the area and the diameter may reflect changes in cell shape, associated with the manifestation of pseudopodia with the neutrophil activation and the implementation of its main effector responses. The value of phase height depends on the characteristics of nuclear structures and granular apparatus of a cell. The volume also characterizes the degree of neutrophil activation, degranulation and vacuolation processes (Vasilenko *et al*., 2008; Galkin and Demidova, 2011; Afonso *et al*., 2012; Beste *et al*., 2015).

The neutrophil activation causes the granules to migrate to the plasma membrane. The exocytosis involves the process of intracellular granules passing through the cortical cytoskeleton and their subsequent fusion with the plasma membrane. The granule membranes become part of the neutrophil plasma membrane, supplying it with receptor proteins, enzymes or components of enzyme complexes. Kay *et al*. (2008) found that the fusion of granule membrane and cytoplasmic membrane results in the significantly increased cell surface area, increasing thereby the cell diameter. In our studies, we had a significant decrease in surface area of neutrophils and no changes in the diameter observed which might indicate a decreased secretory activity of the cells.

Table 2 shows the results of surface protrusion parameters of chicken blood neutrophil when adapting to photojetlag.

The width of the plasma membrane surface protrusions increased significantly on days 16 and 30 by 163.2 and 194.7%, respectively. The height of surface protrusions decreased on day 6 (64.0%), 16 (49.2%) and 30 (43.1%). The depth of the plasma membrane surface

Table 2: The dynamics of surface protrusion parameters of chicken blood neutrophil when adapting to photojetlag

		Cell parameters			
Groups	Days	Surface protrusion width (nm)	Surface protrusion height (nm)	Cell membrane invagination depth (nm)	Cellmembrane invagination width (nm)
1	6	1.9±0.5	53.1±2.5	68.3±4.7	3.3±0.5
	16	1.9±0.5	53.6±2.5	68.6±2.2	3.2±0.6
	30	1.9±0.8	54.2±2.5	68.8±4.2	3.3±0.6
2	6	2.3±0.3	19.1±0.5**	53.8±3.8*	4.8±0.4*
	16	5.0±0.5**	27.0±3.0**	73.3±1.8	4.0±3.0
	30	5.6±0.8**	30.2±2.4**	77.4±5.9	3.2±0.9

Table 3: Functional properties of membrane of blood neutrophils of chickens when adapting to photojetlag

		Cell parameters	
Groups	Days	Elastic modulus (mPa)	Surface potential (mV)
1	6	9.2±0.8	-6.9±1.2
	16	9.2±0.4	-6.8±1.4
	30	9.2±0.6	-6.9±1.1
2	6	1.9±0.5**	-7.7±1.8
	16	2.9±0.6**	-3.2±0.5
	30	3.7±0.5**	-11.9±2.7

Significance of differences compared to the data of the group 1; *p<0.05; **p<0.01

protrusions had a significantly lower value on day 6 (21.2%), their width at the same time significantly exceeded the control value by 45.6%.

Both width and height parameters of the neutrophil surface protrusions may reflect the presence and characteristics of pseudopodia. A moving neutrophil produces many pseudopodia, i.e., their presence also makes it possible to assess the cell motor activity. There are evidences indicating a suppression of spontaneous migration, chemokinesis, chemotaxis and phagocytosis occurring upon neutrophils activation. The literature also bears evidences that the suppression of motor activity of neutrophils during activation occurs due to partial shift of the cytoskeleton structures from musculoskeletal system to the secretory one (Galkin and Demidova, 2011; Rocheleau *et al*., 2015). Otherwise, the reduced surface area and transformed surface protrusions in our studies indicate, apparently, the activation of the cell motor activity.

Studies of the mechanical properties of the neutrophil membrane during the development of various pathological conditions have shown certain relation between the neutrophil membrane stiffness and the degree of their activation (Gainitdinova *et al*., 2015). Table 3 shows the results of the functional of properties chicken blood neutrophil membrane when adapting to photojetlag.

Elastic modulus of the neutrophil membrane characterizes its average rigidity; our studies showed that throughout the entire period of adaptation its value in the test group exceeded significantly the same in the control group by 59-79%. The reduction of elastic

modulus characterizes the increased elasticity and viscosity of the cell membrane and its reduced rigidity in chickens exposed to photojetlag. Rocheleau *et al.* (2015) and Gaspar *et al.* (2015) have shown in their studies that the increase in rigidity of the neutrophil membrane coincides with their activation. The dynamics of parameters of surface area and surface protrusions upon adaptation to photojetlag is consistent with the reduced stiffness of cytoskeleton structures and indicates activation of motor ability of neutrophils.

The analysis of chicken blood leukogram upon their adaptation to photojetlag has revealed changes typical of stress reaction: reduced percentage of lymphocytes and increased number of neutrophils. In these conditions, the neutrophils are the primary elements of the immune defense. Our studies have shown that these conditions help activate the motor activity of neutrophils and suppress their secretory activity.

CONCLUSION

Thus, as a result of the performed studies we found that chronic stress caused by photojetlag leads to the reduction of the surface area of the neutrophils membrane and its rigidity as well as increases the width of surface protrusions with decreasing its height. These changes may indicate the decrease of the secretory activity of neutrophils and activation of their motor ability. Based on the obtained results we may conclude as follows:

- Chronic stress in chickens caused by photojetlag leads to significant and characteristic changes in morpho-functional parameters of blood neutrophils
- The most serious changes occurring upon chicken's adaptation to photojetlag are observed in surface area, the volume, width and height of surface protrusions as well as in the membrane elastic modulus of the neutrophils
- Changes in morphometric parameters of neutrophils of chickens under chronic stress may indicate the decreased secretory activity and the activation of the motor ability of the cells

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