

The State of Micro-Rheological Properties of Red Blood Cells in Rats on the Background of Physical Inactivity

Valery P. Kartashev

Russian State Social University, 129226, Moscow, Russia

ABSTRACT

Objective: to establish changes in the micro-rheological properties of red blood cells in rats under conditions of inactivity. The experiment was undertaken for 34 healthy outbred male rats of six months age. Prior to inclusion in the study, all rats were healthy and did not participate in any earlier studies. Animals were placed in narrow cages for 1 month to minimize their movements. The control group consisted of 32 healthy outbred male rats of a similar age. The work was performed using biochemical, hematological and statistical research methods. In rats under hypodynamic conditions, an increase in the free radical oxidation of plasma lipids and red blood cells was noted. As the duration of hypodynamia in the blood of rats increased, the number of erythrocytes-discocytes decreased, which was most pronounced after a month of observation. This was accompanied by an increase in their blood levels of altered reversibly and irreversibly erythrocyte forms and an increase in spontaneous aggregation of red blood cells. In rats under hypodynamic conditions, a gradual decrease in the level of antioxidant protection of plasma develops. Aggregation readiness and the degree of change in the surface properties of red blood cells increased in these rats. These changes create a risk in animals of increasing morbid burden and weakening of the whole organism in relation to the negative environmental influences

KEY WORDS: RATS, PHYSICAL INACTIVITY, RED BLOOD CELLS, CYTOARCHITECTONICS, AGGREGATION.

INTRODUCTION

Systematic experiments in science allow us to solve various problems of the reaction of the mammals to the influence of the external environment, (Zavalishina, 2018a; Vatnikov et al., 2019). The implementation of any processes in the body is associated with the activation

of biochemical and functional (Usha et al., 2019; Lenchenko et al., 2019) programs under the influence of external factors (Zavalishina, 2018b; Zavalishina, 2018c). This helps the body to adapt, while maintaining viability in any conditions (Skoryatina and Medvedev, 2019; Vorobyeva and Medvedev, 2019). The dynamics of the rheological parameters of blood and especially its shaped elements, which change under many functional conditions and effects on the body, is very important for maintaining the life support of the body (Zavalishina, 2018d; Bikbulatova, 2018a). Red blood cells are a particularly significant element of the microcirculation process, which, by changing their cytoarchitectonics and degree of aggregation, can regulate hemodynamics and metabolism in tissues and, thus, the course of all adaptive processes in the body (Stepanova et al., 2018; Medvedev, 2019).

Article Information: *Corresponding Author: ilmedv1@yandex.ru

Received 08/04/2020 Accepted after revision 21/06/2020

Published: 30th June 2020 Pp-516- 521

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Published by Society for Science & Nature, Bhopal India.

Available at: <https://bbrc.in/>

Article DOI: <http://dx.doi.org/10.21786/bbrc/13.2/24>

It was established that the rheological parameters of red blood cells change against the background of physiological and pathological processes (Zavalishina, 2018e). Moreover, strong effects on the body can worsen the properties of red blood cells and, thus, the microcirculation process in organs, exacerbating the course of pathology (Zavalishina et al., 2019). In the process of studying the body's reactions to various environmental effects on humans (Makhov, Medvedev, 2019), it is difficult to do without evaluating biological processes in an experiment on laboratory animals. Given the importance of erythrocyte rheological parameters for the development of many dysfunctions (Vorobyeva and Medvedev, 2020a) and diseases (Glagoleva and Medvedev, 2020), it is important to study the dynamics of aggregation and cytoarchitectonics of erythrocytes in rats exposed to adverse environmental conditions (Oshurkova and Medvedev, 2018a). This information can serve as a basis for a further search for experimental approaches to optimize the rheological characteristics of red blood cells in conditions of low physical activity of the body, including space flight (Oshurkov and Medvedev, 2018b; Vorobyeva and Medvedev, 2020b). The goal of the present study was set to establish changes in the micro-rheological properties of red blood cells in rats under conditions of inactivity.

MATERIAL AND METHODS

This study was carried out in full compliance with the ethical standards outlined by the European Convention for the Protection of Vertebrate Animals, which are used for experimental and other scientific purposes (adopted in Strasbourg on 03/18/1986 and confirmed in Strasbourg on 06/15/2006). The study took 34 healthy outbred male rats at the age of 6 months. They were placed in narrow individual cells, excluding free movement of animals for 1 month. Prior to inclusion in the study, all rats were healthy and did not participate in the studies.

The control group consisted of 32 healthy outbred male rats at six months of age. Rats were obtained at the age of two months from the laboratory animal nursery of the Branch of the Institute of Bioorganic Chemistry of the Russian Academy of Sciences. Prior to the experiment, the rats were under vivarium conditions in spacious cages (the cell area per rat was 200 cm²). Throughout the time, natural lighting was used, the temperature was maintained at 18–22 °C, the relative humidity was kept at the level of 50–65%. Prior to being taken into the experiment and in its process, all animals received a full-ration ration from compound feed for laboratory animals of the PK-120 brand (Laboratorykorm, Russia). Rats had free access to water throughout the experiment.

RESULTS AND DISCUSSION

The initial values in experimental rats and in the control group were comparable. Under conditions of physical inactivity in rats, an increase in the external manifestations of a deterioration in their general

condition was noted—tarnishing and thinning of the coat, a decrease in interest in the environment, and a decrease in appetite. With an increase in the duration of inactivity in rats, an increase in body weight was found, which amounted to 269.5±4.86 g by the end of the observation. At the same time, a decrease in their endurance level was revealed in the test of forced swimming with weights—after a month of inactivity by 43.9% (table).

In rats in a state of physical inactivity, an increase in the activity level of free radical oxidation processes in plasma lipids was found (the level of acylhydroperoxides and thiobarbituric acid-active products increased by 29.4% and 25.1%, respectively) with a decrease in antioxidant activity by 27.6%. Comparable changes in lipid peroxidation in experimental rats were noted in red blood cells – the number of acyl hydroperoxides and malondialdehyde in them increased. After 4 weeks of inactivity, their number in rats prevailed over the outcome by 46.1% and 43.6%, respectively. The activity of erythrocyte catalase and superoxide dismutase in rats decreased by 24.6% and 20.4% in 4 weeks of inactivity, respectively (Table 1).

Under conditions of physical inactivity in the blood of experimental rats, a decrease in the level of erythrocyte-discocytes to 71.4±0.17% after a month of observation was observed, which led to a gradual increase in their level of changed reversibly and irreversibly erythrocyte forms by 57.9% and 2.1 times. In rats under conditions of physical inactivity, a gradual increase in erythrocyte aggregation was revealed with an increase in their number in the composition of the aggregates and the number of aggregates themselves with a decrease in the blood level of unaggregated red blood cells (229.2 ± 0.29), compared with the level of control and outcome (table).

Any indicators of the body that are significant for its viability respond to the influence of adverse factors from the external environment (Mal et al., 2018). Of great importance in this is the reaction to the action of the hemostatic and rheological characteristics of the blood (Vorobyeva, Medvedev, 2018). It is they who largely determine the volume of tissue perfusion, and, consequently, their trophic (Bikbulatova, 2018b; Boldov et al., 2018). For successful microcirculation, the parameters of the shaped elements, which are controlled by the vessel walls (Zavalishina, 2018f) and lipid peroxidation processes (Karpov et al., 2018), are very important.

It was found that in rats under conditions of physical inactivity, the antioxidant activity of plasma weakens, leading to an increase in the level of acyl hydroperoxides and thiobarbituric acid-active products in it. High lipid peroxidation in plasma damages the walls of blood vessels and the outer surfaces of erythrocyte membranes, negatively affecting their state (Vorobyeva et al., 2018). Moreover, in red blood cells of rats experiencing physical inactivity, the antioxidant defense weakens, which stimulates lipid peroxidation processes in them. High

lipid peroxidation in the plasma and in the erythrocytes violates the structural and functional characteristics of membranes and protein of the cytoskeleton of red blood cells. With increased peroxide oxidation of lipids in erythrocytes occurs the weakening of the synthesis

of ATP, lowering the activity of ion pumps that in these circumstances, no longer cope with the removal of the erythrocyte cytoplasm of excess Ca^{2+} and Na^{+} and are unable to maintain an optimum level of K^{+} (Mal et al., 2018).

Table 1. Showing some haematological parameters of the rats under investigation compared with well Matched controls Legend: reliability of differences in indicators between control and aging rats - * <0.05 ; ** - $p<0.01$

Indicators	Rats in conditions of physical inactivity, $M \pm m$			Control, $n=32$, $M \pm m$
	Exodus, $n=34$	2 weeks, $n=34$	4 weeks, $n=34$	
Body mass, g	232.4 \pm 3.26	248.5 \pm 4.27	269.5 \pm 4.86*	232.4 \pm 4.96
Swimming time, s	158.3 \pm 2.16*	136.2 \pm 3.32*	110.0 \pm 3.04**	161.2 \pm 3.62
Acyl hydroperoxides plasma, $D_{233}/1$ ml	1.46 \pm 0.016	1.71 \pm 0.012*	1.89 \pm 0.025**	1.45 \pm 0.009
Thiobarbituric acid l products, $\mu\text{mol}/$	3.38 \pm 0.018	3.97 \pm 0.019*	4.23 \pm 0.027**	3.42 \pm 0.014
Antioxidant activity, %	36.0 \pm 0.30	31.3 \pm 0.24	28.2 \pm 0.23*	35.1 \pm 0.07
Erythrocyte acyl hydroperoxides, $D_{233}/10^{12}$ erythrocyte	2.82 \pm 0.017	3.52 \pm 0.012*	4.12 \pm 0.024**	2.82 \pm 0.015
Erythrocyte malondialdehyde, $\text{nmol}/10^{12}$ erythrocyte	1.10 \pm 0.013	1.37 \pm 0.011*	1.58 \pm 0.012**	1.11 \pm 0.010
Erythrocyte catalase, $\text{ME}/10^{12}$ erythrocyte	8850.0 \pm 12.9	8000.0 \pm 13.6*	7100.0 \pm 19.8**	8960.0 \pm 16.2
Erythrocyte superoxide dismutase, $\text{IU}/10^{12}$ erythrocyte	1590.0 \pm 7.16	1470.0 \pm 8.23*	1320.0 \pm 6.35**	1650.0 \pm 13.15
Discocytes, %	84.2 \pm 0.14	77.1 \pm 0.16*	71.4 \pm 0.17**	84.1 \pm 0.14
Reversibly altered red blood cells, %	9.5 \pm 0.12	11.8 \pm 0.07*	15.0 \pm 0.09**	9.5 \pm 0.11
Irreversibly altered red blood cells, %	6.3 \pm 0.07	11.1 \pm 0.13**	13.6 \pm 0.12**	6.4 \pm 0.12
The amount of red blood cells included in the aggregates	31.2 \pm 0.15	37.2 \pm 0.14*	42.6 \pm 0.08**	30.2 \pm 0.06
Number of units	6.3 \pm 0.09	7.0 \pm 0.12*	8.2 \pm 0.08**	6.1 \pm 0.05
The number of free red blood cells	292.3 \pm 0.18	257.0 \pm 0.26*	229.2 \pm 0.29**	292.8 \pm 0.22

In these circumstances, in the blood of rats develops a gradual increase in the number of red blood cells that do not have a biconcave shape. The resulting changes in the red blood cells ensures the growth of content in their blood reversible and irreversible changes of their species. So, the animals in the conditions of inactivity by increasing the level of red blood cells in a state of echinocytosis with very variable form and changed to stomatita having the form of a one-sided full drive. Further transformation of these red blood cells rapidly

leads to the appearance of steroidica, serotoninocytes and in the end of spherocyte, which in a short time is lysed.

Found in rats in conditions of hypodynamy increased aggregation of red blood cells is provided by the emerging changes in the magnitude of the charge of their membranes due to active degradation on the surface part of glycoproteins as a result of the excess products of lipid peroxidation. Increased synthesis of reactive

oxygen species in rats in conditions of hypodynamia causes oxidative alteration of erythrocyte membranes and plasma damage to plasma proteins, having the ability to link with each other erythrocytes in the process of aggregation. In addition, the increasing lipid peroxidation in plasma and in red blood cells impairs the ability of erythrocytes to disaggregate due to the large force of red blood cells in aggregates and increase the speed of the process (Makhov, Medvedev, 2018).

The increase in the number of free aggregates in rat blood under conditions of physical inactivity contributes to damage to the vascular endothelium, which contributes to greater contact of the subendothelium and blood with activation of hemostasis due to this process, significantly worsening hemodynamics in the capillaries (Bikbulatova, 2018a). An increase in the number of aggregates in the blood of experimental rats can impede blood flow in parts of small vessels and in their vasa vasorum, which causes degeneration of the vessel walls and weakening of the synthesis of disaggregants in the vessels, providing control over the aggregation of red blood cells (Zavalishina, (2018e; Zavalishina et al., 2019).

CONCLUSION

In rats under hypodynamic conditions, a gradual weakening of the plasma antioxidant defense occurs. This is accompanied in rats by an increase in its levels of lipid peroxidation products. As a result, the alteration of the outer membranes of red blood cells is enhanced in animals, increasing the number of their altered forms. In rats under conditions of inactivity, the ability to aggregate also increased and the number of red blood cells in the blood increased. This is very important for reducing the resistance of animals to animals with low physical activity, which makes them very sensitive to the influence of any negative environmental factors.

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