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Research Article

Physiological changes in the microrheological properties of erythrocytes with age in animals

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ABSTRACT

The aim: determine age-related changes in the micro-rheological characteristics of erythrocytes in rats in the late stages of ontogenesis.

Materials and methods. The study took 96 healthy outbred male rats, including 34 animals at the age of 18 months, 30 animals at the age of 24 months and 32 heads at the age of 30 months. Before inclusion in the study, all rats were healthy and did not participate in the studies. The control group consisted of 31 healthy outbred male rats at six months of age. The study was conducted using biochemical, hematological and statistical research methods.

Results. In aging rats, an increase in the level of activity of free radical oxidation processes in plasma lipids and erythrocytes was found. With an increase in the chronological age in the blood of the observed rats, a decrease in the level of erythrocytes-discocytes was noted, which was most pronounced in rats at 30 months of age. This was accompanied by an increase in their level of reversibly and irreversibly altered erythrocyte forms and an increase in spontaneous aggregation of erythrocytes.

Conclusions. In rats during aging, a gradual decrease in plasma antioxidant activity was found. During aging in rats, the aggregation readiness and the degree of change in the surface properties of erythrocytes progressively increase. This is of great importance for the increase with aging of the morbid burden and the weakening of the whole organism in relation to the impact of negative environmental influences.

Keywords: Rats, Aging, Red blood cells, Cytoarchitecture, Aggregation.

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INTRODUCTION

The steady development of biological science has still not made it possible to solve many of the problems of the ontogenetic development of the mammalian organism[1]. It is clear that the deployment of a sequence of phenomena of individual development is associated with the implementation of a genetic[2] program under the influence of environmental factors[3,4]. This allows age-related changes to affect all tissues of the body, significantly worsening the functional potential and making it very likely to die[5]. The dynamics of the rheological parameters of the blood and the formed elements of its components, capable of changing under different functional states and external influences on the body, has a serious value for the realization of aging[6, 7]. A very important element of microcirculation are cells - red blood cells, which, changing their cytoarchitecture and aggregation activity, significantly affect the processes of hemodynamics and metabolism in tissues and determine the course of all adaptive processes in the body[8]. It is known that their rheological parameters can change against the background of physiological and pathological processes[9]. It is clear that very often at older ages, these indicators can change in non-physiologically beneficial boundaries, which, apparently, can negatively affect the course of microcirculation in organs, sometimes significantly aggravating the course of pathology[1].

At the same time, in the process of searching for therapeutic approaches for various pathological phenomena in humans[4], it is difficult to do without studying biological processes in experimental models that are mainly carried out in laboratory animals and primarily in rats. Due to the importance of erythrocyte rheological parameters in the development of many dysfunctions, including age-related changes in the blood, it is necessary to develop options for overcoming them. In this regard, it is very important to study the aspects of aggregation and cytoarchitecture of erythrocytes in aging rats. This information can serve as a basis for further research in experimental approaches to optimize the rheological characteristics of erythrocytes at older ages and further very careful consideration of this information in gerontological observations on people[1,5].

Taking into account these circumstances, the aim of the work is to ascertain the age-related changes in the microrheological characteristics of erythrocytes in rats in the late stages of ontogenesis.

MATERIALS AND METHODS

This study was carried out in full compliance with the ethical standards designated 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 15.06.2006). The study took 96 healthy outbred male rats, including 34 animals at the age of 18 months, 30 animals at the age of 24 months and 32 heads at the age of 30 months. Before inclusion in the study, all rats were healthy and did not participate in the studies. The control group consisted of 31 healthy outbred male rats at six months of age. Rats were obtained at the age of two months from the nursery of laboratory animals of the Branch of the Institute of Bioorganic Chemistry of the Russian Academy of Sciences (Moscow Region, Pushchino). The rats were kept in vivarium conditions in fairly large cages (the size of the cage area per rat was 200 cm^2). Natural lighting was used, the temperature was maintained at 18-22 ºC, the relative humidity was kept at the level of 50-65%. All animals All animals were fed on a full ration ration from animal feed for laboratory animals of the brand PK-120 (produced by the company Laboratorkorm, Moscow). Rats had free access to water.

All rats were weighed on an electronic scale brand BM1502M-II (production OKB Vesta, Russia). Their endurance was assessed in the swimming dough using a load of 10% of the body weight of each rat, which was fixed to the base of the tail. Testing of animals was performed in an aquarium with a water depth of 0.8-0.9 m and a water temperature of 24-26ºC. We recorded the time of swimming before the onset of signs of complete fatigue, which manifested itself in the form of cessation of swimming movements and immersion of the rat under water for 10 seconds[10].

In the study, blood from rats was taken from a tail vein. Plasma lipid peroxidation was determined in all rats by estimating the content of thiobarbituric acid-active products using the Agat-Med kit, the number of acyl hydroperoxides with recording the value of plasma antioxidant activity[11]. The erythrocyte level of lipid peroxidation was estimated by the number of malonicdialdehyde and acyl hydroperoxides[12] taking into account the antioxidant potential of catalase and superoxide dismutase[13].

Using an Olympus CX-41 phase-contrast microscope (Olympus, Japan), providing a magnification of 1200 times, red blood cells were divided into discoid, modified reversibly and irreversibly modified[14].

Erythrocyte aggregation was assessed by light microscopy, using a Goryaev camera, in the course of determining the number of erythrocyte aggregates, the values of aggregated erythrocytes that did not enter into aggregation after their washing and resuspension[14]. The digital values obtained were processed using Student's t-test using the program StatSoft STATISTICA for Windows 6.0.

RESULTS AND DISCUSSION

In rats taken as experience, as the chronological age increased, an increase in the external manifestations of aging was revealed - the wool became thinner and thinned, their physical activity and appetite decreased in rats, and the interest in the surrounding reality weakened. During the growth of chronological age in aging rats, a regular increase in body weight was found, amounting to 379.8±7.18 g in 30 monthly rats. At the same time, a gradual decrease in their endurance level was observed during the test of forced swimming with the burdening of 30 month-old rats compared with 18 months by 54.7% and 80.4% compared with the control.

In aging rats, an increase in the level of activity of free radical oxidation processes in plasma lipids (the level of acyl hydroperoxides and thiobarbituric acid-active active products increased by 21.6% and 20.9%, respectively) was found, while the antioxidant activity decreased by 28.0%. When compared with the control values, the levels of acylhydroperoxides and thiobarbituric acid-active products of rats aged 30 months were increased by 30.8% and 26.1%, respectively, while their antioxidant activity was lower than in the control group by 35.8 % (table).

Comparable changes in lipid peroxidation in rats taken in the study were noted in erythrocytes - the number of acylhydroperoxides and malondialdehyde in them increased. Their number in 30 month-old rats prevailed compared with 18 month-olds by 34.9% and 40.2%, respectively. At the same time, they exceeded - control values by 40.1% and 46.4%, respectively. The activity of erythrocyte enzymes catalase and superoxide dismutase in aging rats as a whole decreased by 22.2% and 20.4%, respectively. At the same time, it was inferior in 30 monthly rats to the level of control by 23.9% and 21.2%, respectively (table).

In the course of increasing the chronological age in the blood of the observed rats, a decrease in the level of erythrocytes-discocytes was noted to 70.0 ± 0.14% in rats at 30 months of age, which led to a gradual increase in their level of reversible and irreversible erythrocyte forms (in 30 month-old compared with the control group, respectively, by 55.2% and 2.1 times).

In rats taken into the experience of rats, as the chronological age increased, the ability to aggregate erythrocytes increased with their total inclusion in the aggregates and the number of aggregates while the level of non-aggregated erythrocytes decreased (225.5±0.22) compared with the control level (table).

Various indicators of the organism that realize its viability strongly depend on the characteristics of its heredity and the effect of external factors on it[15]. Of great importance in this is the response to the current conditions of the hemostatic and rheological characteristics of the blood[16], which determine the amount of nutrients and oxygen entering the cells[17]. Especially important for the success of microcirculation are the features of the formed elements that are affected by the walls of blood vessels[18] and the influence of lipid peroxidation processes in the blood[19].

It has been established that during aging in rats the plasma antioxidant activity weakens, leading to an increase in the level of acyl hydroperoxides and thiobarbituric acid-active products. Excessive lipid peroxidation in plasma damages the walls of blood vessels and receptors on the membranes of blood cells, including red blood cells, negatively affecting their state [20]. At the same time, antioxidant protection weakens in erythrocytes, which enhances lipid peroxidation processes in them.

Excessive lipid peroxidation in plasma and erythrocyte membranes violates the structural and functional characteristics of the membranes and protein cytoskeleton of the red blood cells.

Against the background of enhanced lipid peroxidation in erythrocytes, a weakening of adenosine triphosphate synthesis occurs, reducing the activity of ion pumps, which under these conditions can no longer cope with the release of increasing Ca^{2+} and Na^{+} influx and maintaining the K^{+} level optimum[21,22]. During the increase in Ca^{2+} level in erythrocytes, K^{+} decrease and Na^{+} level growth, the volume of water decreases and the hemoglobin concentration increases, which changes the shape of a certain number of red blood cells. Obviously, the disturbance of cytoarchitectonics is associated with changes in the structure of spectrin due to the activation of lipid peroxidation and a decrease in the distance between spectrin molecules. Under these conditions, the surface area of the inner part of the lipid bilayer decreases and echinocytes are formed. Also under these conditions, the phospholipid membrane complex changes with the appearance of protein-free zones, which quickly combine. An excess of Ca^{2+} appears with some polar acid-phosphate groups in the lipid bilayer and leads to the formation of their structural bonds. Ultimately, this leads to a decrease in the inner part of the lipid membrane and the formation of a spheroehinocyte from an erythrocyte[23].

Under these conditions, a gradual increase in the number of red blood cells that do not have a biconcave shape develops. Emerging changes in red blood cells provides an increase in blood levels of reversibly and irreversibly modified their varieties. So, in animals at the age of 30 months, the level of erythrocytes significantly increases, echinocytosis in spheres with spikes of variable form on their surface and by stomacytosis to the unilaterally arched state of the spikes has undergone. Further transformation of such erythrocytes quickly leads to the appearance of spheroehinotsit, spherotomatocyte and necessarily spherocyte, capable of lysing in a short time. These changes inhibit the course of hemocirculation through the vessels and, above all, through the capillaries. The difficulties of microcirculation are caused by the fact that during the passage through the capillaries all red blood cells must experience elongation and take an ellipsoidal shape. This ability is greatest in discocytes. Only they can be extended from 8 to 17 microns. At the same time, the spheroehinocyte can be extended only from 5.5 to 8 µm, and the erythrocyte spherotomatocyte is extended from 5.5 to 7µm[24]. In the blood, erythrocytes-spherocytes constantly experience rotation and manifest themselves as very hard particles. In this regard, their rheological properties are worst than those of all other red blood cells[25,26].

The increase in the activity of erythrocyte aggregation found in rats during aging is strongly provided by the resulting changes in the charge level on their membranes due to the degradation of a certain amount of glycoproteins on them by the action of an excess of lipid peroxidation. Strengthening the synthesis of reactive oxygen forms in aging rats creates conditions for oxidative membrane alteration and damage to plasma plasma proteins, which have the ability to adhere red blood cells as “bridges” during their aggregation. Increasing lipid peroxidation in plasma and erythrocytes increases the threshold of the process of their disaggregation by increasing the connection of red blood cells into aggregates, increasing the speed of this process due to oxidative damage to their membranes[5].

Apparently, the increase in the intensity of aggregation of erythrocytes found during aging in rats is significantly enhanced by the increased influence of catecholamines, the content of which increases in the event of any ill-being in the body and against the background of aging. Under conditions of α1 receptor activation, the main messengers are the Ca^{2+}calmodulin system and the “chain” of phosphatidylinositol transformations. An increase in the level of α_2-adrenoreceptor activity leads to a weakening of adenylatecyclase against the background of influences from receptors on Gi proteins, lowering the level of cyclic adenosine monophosphate in the cell, which causes Ca^{2+} to enter it and enhances erythrocyte aggregation[8].

An increase in the number of free aggregates in the blood of rats with age resulted in damage to the vascular endothelium, which contributed to the contact of the subendothelium and blood and the activation of hemostasis, which significantly worsened the blood microhreology in the capillaries[10]. Increasing the level of aggregates freely moving through the vessels can block a certain amount of vasa vasorum, which leads to dystrophy in the walls of blood vessels and weakens the synthesis of substances in vessels that provide hemostatic control and control of erythrocyte aggregation[13,20].

CONCLUSION

In rats during aging, a gradual decrease in plasma antioxidant activity was found. This is accompanied by an increase in the level of lipid peroxidation products in it. The developing situation causes the alteration of the outer membranes - erythrocytes, which adversely affects their functions. During aging in rats, the aggregation readiness and the degree of change in the surface properties of erythrocytes progressively increase. This is of great importance for the increase with aging of the morbid burden and the weakening of the whole organism in relation to the impact of negative environmental influences.

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Table. Characteristics of the examined rats

Registered indicators	Aging rats, n=96, M±m			Control, n=31, M±m
	18 months of observation, n=34	**24 months of observation, n=30**	**30 months of observation, n=32**	
Bodyweight, g	334.1±8.24**	356.7±8.92**	379.8±7.18**	233.1±6.83
Swimming time, s	139.6±3.24*	114.7±4.73**	90.2±6.23**	162.7±4.97
Plasma acylhydroperoxide, D_{233}/1 ml	1.57±0.019	1.84±0.028*	1.91±0.046**	1.46±0.008
Thiobarbituric acid products, μmol/l	3.63±0.019	4.10±0.036*	4.39±0.029**	3.48±0.012
Antioxidant activity, %	32.9±0.36	28.4±0.28	25.7±0.27*	34.9±0.009
Erythrocyte acylhydroperoxide, $D_{233}/10^{12}$erythrocyte	2.95±0.016	3.46±0.024*	3.98±0.015**	2.84±0.017
Erythrocyte malondialdehyde, nmol/10^{12}erythrocyte	1.17±0.011	1.42±0.008*	1.64±0.007**	1.12±0.004
Red Blood Catalase, ME/10^{12} erythrocyte	8800.0±13.2	8000.0±18.5*	7200.0±22.8**	8920.0±14.5
Erythrocyte superoxide dismutase, ME/10^{12}эр. erythrocyte	1590.0±12.70	1500.0±8.16*	1320.0±12.27**	1600.0±14.02
Discocytes, %	83.4±0.10	76.3±0.16*	70.0±0.14**	83.8±0.13
Reversibly modified red blood cells, %	9.7±0.12	12.8±0.04*	14.9±0.10**	9.6±0.08
Irreversible red blood cells, %	6.9±0.09	10.9±0.16**	15.1±0.12**	7.1±0.14
The amount of red blood cells that are included in the units	32.6±0.12	38.5±0.10*	44.7±0.16**	30.3±0.08
Number of units	6.2±0.05	7.0±0.12*	8.3±0.11**	6.0±0.07
The number of free red blood cells	288.5±0.25	244.2±0.34*	225.5±0.22**	294.5±0.26

Legend: the reliability of differences in performance between control and aging rats– *<0,05; ** – p<0,01.