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Physiological Features Of Erythrocytes In Rats At Late Stages Of Ontogenesis.

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ABSTRACT

Being the leading component of microcirculation, erythrocytes through their cytoarchitectonics and ability to aggregate largely determine the hemodynamic and metabolic tissue homeostasis at the level of the capillary bed and influence the realization of many adaptive reactions of the body. To search for various therapeutic effects in many pathological conditions in man, it is impossible to do without the use of various experimental models, conducted in an overwhelming number of cases on laboratory rats. Considering the importance of rheological properties of erythrocytes in the development of age-associated thrombophilia and the need to develop approaches to its elimination, the study of the state of erythrocytes in aging rats becomes urgent. The aim of the work is to establish the features of the age-related dynamics of the micro rheological properties of erythrocytes in aging rats. 95 healthy Wistar male rats were examined, including 32 rats aged 18 months, 29 animals aged 24 months and 34 rats of 30 months of age. Biochemical, hematological and statistical methods of investigation has been applied. The control was represented by 27 healthy male Wistar rats at the age of 6 months. It was found that in healthy aging rats the micro rheological properties of erythrocytes gradually worsen with an increase in the content of their altered forms in the blood and in the number of their circulating aggregates. This makes a significant contribution to the growing morbidity with age, the sensitivity of the organism to the negative influences of environmental factors and contributes to the realization of a hereditary predisposition to various pathologies.

Keywords: aging, rats, erythrocytes aggregation, surface geometry.

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INTRODUCTION

At the present stage of development of biology, there remains an urgent need for further study of various aspects of the functional state of the mammalian and human organism in norm and pathology [1, 2]. Much attention is paid to the rheological characteristics of blood and its uniform elements, including in the age aspect, under conditions of various pathologies, as well as against various influences on the organism [3, 4].

Being the leading component of microcirculation, erythrocytes through their cytoarchitectonics and ability to aggregate largely determine the hemodynamic and metabolic homeostasis of tissues at the level of the capillary bed and influence the realization of many adaptive reactions of the organism [5, 6]. In this case, the rheological properties of erythrocytes are able to change under physiological, borderline and pathological conditions [7, 8].

Based on the genetic and environmental components, the aging process inevitably affects all body systems, progressively worsening their functioning and making its death more and more likely [9, 10]. The red blood cells, which play the main role in the functioning mechanisms of the whole organism, are not an exception [11, 12]. Of particular relevance is the evaluation of various aspects of theology in the clinic [13, 14], where the success of the treatment of the underlying disease, the effectiveness of prevention and therapy of its complications and the overall prognosis depend on its timely and accurate assessment and the effectiveness of its correction [15]. At the same time, it is impossible to avoid the use of various experimental models, which are presented in an overwhelming number of cases on laboratory rats, in order to search for various therapeutic effects in many pathological conditions in humans [2]. Considering the importance of rheological properties of erythrocytes in the development of age-associated thrombophilia and the need to develop approaches to its elimination, the study of the state of erythrocytes in aging rats becomes urgent. The data obtained can serve as a basis for subsequent search in the experiment approaches to optimize the state of erythrocytes in later ages, followed by careful data transfer to erotological studies [16].

In this connection, the goal of the work is to establish the features of the age-related dynamics of the micro rheological properties of erythrocytes in aging rats.

MATERIALS AND METHODS

The research was conducted in strict accordance with ethical principles established by the European Convent on protection of the vertebrate used for experimental and other scientific purposes (adopted in Strasbourg in March, 18th, 1986, and confirmed in Strasbourg in June, 15th, 2006) and approved by the local Ethics Committee of Russian State Social University (Record №16, dated December, 7th, 2015).

95 healthy Wistar male rats were monitored, including 32 rats aged 18 months, 29 animals aged 24 months and 34 rats of 30 months of age. Until the moment of taking into the study, the rats were not in any experiments and did not tolerate any diseases. The control is represented by 27 healthy male Wistar rats at the age of 6 months.

In all animals, the intensity of peroxide oxidation of plasma lipids was estimated from the concentration of Thiobarbituric acid-active products by the "Agat-Med" kit, acyl hydro peroxides, and the antioxidant potential of the liquid part of the blood [17].

In the erythrocytes, the level of lipid peroxidation (LPO) by the amount of malonicdialdehyde and acyl hydro peroxides, as well as the activity of catalase and superoxide dismutase, was determined by traditional methods [17].

During light phase-contrast microscopy, erythrocytes were divided into discocytes, reversibly altered and irreversibly altered forms with the calculation: index of transformation = percentage of reversibly altered erythrocytes + percent of irreversibly changed red blood cells / percentage of discoid erythrocytes; index of reversible transformation = percentage of reversibly altered erythrocytes / percentage of discoid erythrocytes; index of irreversible transformation = percentage of irreversibly changed red blood cells / percentage of discoid red blood cells and index of reversibility = percentage of reversibly altered red blood cells / percent of irreversibly altered red blood cells.

Aggregation activity of erythrocytes was recorded with the help of a light microscope in the Goriaev chamber in terms of the number of erythrocyte aggregates, the number of aggregated and non-aggregated erythrocytes in a suspension of washed erythrocytes [6]. In this case, the average size of the aggregate was calculated = the sum of red blood cells that fell into aggregates / the number of erythrocyte aggregates. The aggregation index was determined = (the average size of the aggregate x the number of erythrocyte aggregates + the number of free red blood cells) / (the number of erythrocyte aggregates + the number of freely lying red blood cells and the percentage of unaggregated erythrocytes = (number of freely lying erythrocytes x 100) / (average aggregate size x number of erythrocyte aggregates + number of freely lying red blood cells).

Statistical processing of the obtained data was carried out by Student's t-test.

RESULTS AND DISCUSSION

In the observed rats, as the age increased, there was an increase in the characteristic external signs of aging - tarnishing of the wool, its thinning, decrease in activity and appetite in animals, lack of interest in the surrounding, pallor of visible mucous membranes.

An increase in the activity of free radical lipid oxidation in the liquid part of the blood (in 18 months acyl hydro peroxides 1.60±0.024 D_{233}/1 ml, Thiobarbituric acid-active products 3.80±0.016 umol/l, at2,5 years 1.87±0.058 D_{233}/1 ml and 4.28±0.032 umol/l, respectively) with a decrease in antioxidant activity from 30.7±0.32% at 18 months, up to 24.4±0.29% at 30 months). Similar values in the control were 1.44±0.007 D_{233}/1 ml, 3.46±0.016 umol/l and 34.8±0.010%, respectively.

As the age increased, the content of acyl hydro peroxides and malonicdialdehyde in erythrocytes increased by 11.6% and 12.8%, respectively, in aging rats. At the same time, the activity of erythrocyte catalase in the oldest observed rats was 8830.0±25.6 ME/10^{12} erythrocytes, and the erythrocyte SOD of 1500.0±17.12 ME/10^{12} erythrocytes, significantly less than the values of the control group (Table).

Table: Thrombolytic activity in aging rats

Indicators	Aging rats, n=95, M±m			Control, n=27, M±m
	18 months, n=32	24 months, n=29	30 months, n=34	
Acyl hydro peroxides of erythrocytes, D_{233}/10^{12} erythrocytes	3.43±0.06*	3.68±0.12**	3.83±0.16**	2.94±0.12
Malonicdialdehyde of erythrocytes, nmol/10^{12} erythrocytes	1.56±0.04*	1.66±0.03**	1.78±0.07**	1.22±0.08
Catalase of erythrocytes, ME/10^{12} erythrocytes	9310.0±24.7*	9110.0±18.3*	8830.0±25.6**	10210.0±22.7
Superoxide dismutase of erythrocytes, ME/10^{12} erythrocytes	1580.0±10.66*	1540.0±8.64*	1500.0±17.12**	1670.0±6.83
Erythrocytes-discocytes,%	78.0±0.12*	75.2±0.08*	74.0±0.14**	85.8±0.19
Reversibly modified erythrocytes,%	13.1±0.03*	13.5±0.08**	14.0±0.10**	9.8±0.13
Irreversibly modified erythrocytes,%	8.9±0.08**	11.3±0.06**	12.0±0.03**	4.4±0.14
value of the transformation index	0.28±0.002**	0.33±0.004**	0.35±0.008**	0.16±0.005
value of the reversible transformation index	0.17±0.006**	0.18±0.005**	0.19±0.006**	0.11±0.003
value of the irreversible transformation index	0.11±0.007**	0.15±0.004**	0.16±0.004**	0.05±0.004

value of the reversibility index	1.47±0.015**	1.19±0.013**	1.17±0.010	2.22±0.01
total number of red blood cells included in the aggregates	37.5±0.15*	39.0±0.10*	42.4±0.16**	32.4±0.08
Numberoferythrocyteaggregates	7.5±0.08*	7.8±0.07**	8.4±0.04**	6.3±0.05
number of freely lying red blood cells	249.7±0.25**	239.1±0.22**	231.0±0.16**	282.4±0.21
Aggregation value	1.11±0.06	1.13±0.10	1.14±0.07	1.09±0.07
value of the percentage of non-aggregated erythrocytes	87.0±0.11	85.9±0.08	84.6±0.03	89.8±0.14
value of the average size of the aggregate, cells	5.0±0.07	5.0±0.03	5.0±0.06	5.1±0.06

Legend: the reliability of the differences between the control and aging rats - * <0.05; ** - p <0.01.

The maximum decrease in discocytes (74.0±0.14%) was observed in the blood of 30 monthly rats, compared with the control (85.8±0.19%), which was accompanied by an increase in the number of reversible and irreversibly altered forms of 42.8% and 2.72 times against control. In this case, the aging rats showed a gradual increase in the index of transformation, the index of reversible transformation and the index of irreversible transformation with a decrease in the index of reversibility.

Growing aggregation capacity of erythrocytes was noted in aging rats with an increase in their total involvement in aggregates and the number of erythrocyte aggregates with a decrease in the number of free erythrocytes (231.0±0.16), in comparison with the control. The percentage of non-aggregated erythrocytes in the course of aging in rats by 30 months. Yielded to the control values by 6.1% when the average size of the aggregate was comparable in both groups of children.

In the study, it was found that as the aging progresses, the antioxidant potential of the plasma is weakened in rats, resulting in an increase in the amount of acyl hydro peroxides and Thiobarbituric acid products and deterioration in metabolism in tissues [18]. In addition, the activation of lipid peroxidation in the plasma causes alteration of the vascular endothelium and surface structures of blood cells, including the most abundant population of erythrocytes, thereby negatively affecting their functions [19]. This is accompanied by a decrease in the antioxidant protection of the red blood cells themselves, with the activation of lipid peroxidation therein [20].

The situation that is created in many respects favors the loss of a part of the red blood cells as they grow older in the biconcave form, which complicates the process of their movement through the vessels in the microcirculation basin [21,22]. The resulting changes in erythrocytes lead to an increase in the blood of reversibly and irreversibly altered varieties [23]. Thus, in rats at 30 months, the number of erythrocytes transformed by echinocytosis into spheres covered with spines of various shapes, and by means of dermatocytosis to a unilaterally curved disc increases to the maximum. Further transformation develops to the spheroechinocyte, spherostomatocyte, and eventually to the spherocyte, which precedes cell destruction [24,25].

The increase in erythrocyte aggregation found in old rats is largely due to the resulting changes in the charge of their membranes, due to the degradation of glycoproteins that have a negative charge under the action of intense LPO. The increase in the generation of active oxygen forms under these conditions provides for oxidative alteration of membrane structures in aging rats, with simultaneous damage to globular plasma proteins capable of connecting as "bridges" between individual red blood cells and realizing the process of their aggregation [26]. Under these conditions, the products of lipid peroxidation increase the threshold of erythrocyte disaggregation by increasing the adhesion of erythrocytes in aggregates, increasing the speed of the aggregation process against the background of oxidative damage to the lipids of their membranes [27,28].

The revealed increase in erythrocyte aggregation in aging rats is largely due to the effect of catecholamines, the concentration of which often increases significantly with aging [29,30]. When α1-receptors are activated, the Ca^{2+}-calmodulin system acts as an intermediary with involvement of intracellular reactions of phosphatidylinositol in the cascade [31]. Activation of α_2-adrenergic receptors is realized by inhibition of adenylatecyclase due to the influence of receptor agonist on GI proteins, leading to an increase in the amount of Ca^{2+} in the cell, which additionally increases erythrocyte aggregation [32].

The increase in the number of aggregates moving freely in the blood of the aging rat's leads to damage to their endothelial lining, causing the exposure of sub endothelial structures, which "triggers" homeostasis, significantly worsening the process of blood theology [33]. Numerous freely circulating aggregates are capable of blocking part of the vase vasorum, which significantly weakens the vascular control over the process of erythrocyte aggregation, as a result of a decrease in the production of the disaggregants in the endothelium.

CONCLUSION

In healthy aging rats, the aggregation activity of erythrocytes gradually increases, which is accompanied by an increase in the content of altered forms of erythrocytes in their blood, inevitably leading to an increase in the number of circulating aggregates of various sizes. This contributes to the morbid weighting that is observed with age, increasing the sensitivity of the organism to the negative influences of environmental factors and promoting the realization of a hereditary predisposition to various, primarily, vascular pathologies.

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