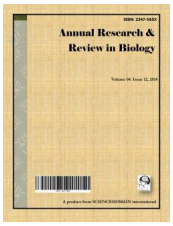

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Physiological Dynamics of Platelets' Activity in Aged Rats

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

The process of aging inevitably touches the system of hemostasis and leads very often to the development of thrombophilia. Platelets are an important component of hemostasis as they mostly determine its activity on the whole. It's very interesting to estimate the dynamics of platelet activity in rats as they are important objects of laboratory investigations in the period of their bodies' evident aging – at the age between 18 and 30 months of life. The aim of the study is to establish the peculiarities of age-specific dynamics of platelet activity in aged rats. There were examined 95 healthy Wister male-rats, including 32 rats at the age of 18 months, 29 rats at the age of 24 months and 34 rats at the age of 30 months. The control group was composed of 27 healthy male-rats of Wistar line at the age of 6 months. We applied biochemical, hematological and statistical methods of investigation. Between 18-30 months of life the rats were noted to have rise of acylhydroperoxides' content in plasma on 19.9%, thiobarbituric acid-active products – on 18.9% and lowering of plasma antioxidant activity on 23.8%. Between 18-30 months of life healthy rats were found to have gradual acceleration of platelet aggregation (platelet aggregation with ADP at the age of 18 months was equal to 38.4±0.12s, at the age of 30 months – 33.1±0.16s), content increase of platelets' active forms in their blood (disco-echinocytes at the age of 18 months – 14.0±0.18%, at the age of 30 months – 17.5±0.17%) and number increase of their freely circulating aggregates (small aggregates at the age of 18 months – 3.5±0.09, at the age of 30 months – 7.2±0.13 on 100 freely lying

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platelets). Found increase of platelet activity in rats contributes greatly to their rising with age morbid aggravation, increase of their bodies' sensitivity to negative impacts of environmental factors and promotes the realization of hereditary predisposition to different pathology. Received results can be useful for future researches connected with formation aspects of age-specific thrombophilia and can be taken into account at estimation of different variants of life's experimental prolongation.

Keywords: Aging; rat; platelet aggregation; intravascular activity.

1. INTRODUCTION

The process of aging realizes slowly but inevitably. It touches all the systems of the internals, progressively worsens their functioning and makes the death of a body more and more possible [1]. The system of hemostasis is not an exception including platelets which play the main role in the initiation mechanisms of its functioning [2]. The estimation of various aspects of platelet functions is especially urgent in clinic where the success in treatment of the main disease and the common prognosis [3] often depend on timely and exact estimation of platelet hemostasis activity [4] and efficiency of its correction. At the same time, it's impossible to do without application of various experimental models for the search of different therapeutic impacts at many pathological states of a man. The formation of these models is mostly conducted on gnawing animals including rats [5]. Taking into consideration the importance of hemostasis platelet section in the development of age-specific thrombophilia and the necessity of working out of approaches to its removal it becomes clear that studying of blood corpuscles' activity in aged rats [6] is really urgent. Received data can serve the foundation for the consequent experimental search of approaches to optimization of platelet activity in late ages with consequent cautious transfer of received data into gerontological researches on human beings.

So, we put the following aim in the present study – to establish peculiarities of age-specific dynamics of platelet activity in aged rats.

2. MATERIALS AND METHODS

All the investigations in the present work were conducted in full correspondence with ethical norms and recommendations on humanization of work with laboratory animals containing "The European Convent on the protection of vertebrate animals used for experiments or in other scientific purposes" (Strasbourg, 1986). Given research was approved by local Ethics Committee of Russian State Social University on May, 14th, 2015 (record №5).

We observed 95 Wister male-rats including 32 rats at the age of 18 months, 29 rats at the age of 24 months and 34 rats at the age of 30 months. Before being taken into the research the rats have participated in no experiments and have suffered no diseases. The control group was composed of 27 healthy Wister male-rats at the age of 6 months.

The intensity of plasma lipid peroxidation (LPO) in all the animals was estimated according to the concentration of thiobarbituric acid (TBA)-active products by a set "Agat-Med", acylhydroperoxides (AHP) with determining of the antioxidant potential value of liquid part of blood [7]. Determination of platelet concentration in capillary blood was conducted in Gorjaev's box. Aggregation of platelets (AP) was determined by visual micromethod with some inductors: ADP (0.5×10^{-4} M), collagen (dilution 1:2 of the basic suspension), thrombin (0.125 un/ml), ristomicin (0.8 mg/ml), adrenaline (5×10^{-6} M) and hydrogen peroxide (7.3×10^{-3} M), and also combinations of ADP with adrenaline, ADP with collagen and adrenaline with collagen [8]. Intravascular activity of platelets (IAP) was estimated with the phase contrast [8]. Statistical analyses of the collected data were conducted using the programs: "Statistics for Windows v. 6.0" and "Microsoft Excel". Significances of the differences were assessed using Student's t test. P values < 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

While aging the observed rats were found to have intensification of typical aging external evidence – dead and sparse hair, reduction of activity and appetite in animals, absence of interest to the environment, paleness of discernible mucous membranes.

The observed rats were noted to have some activity rise of free-radical lipid oxidation in liquid

part of blood against the background of aging (between 18-30 months AHP increased on 19.9%, TBA-active products increased on 18.9%) at lower AOA between 18-30 months of life on 23.8%. These values of 2.5 years' experimental rats differed from control ones on 29.8%, 23.7% and 31.8%, respectively.

The quantity of platelets in blood of all the observed rats corresponded to the norm. AP in aged animals accelerated with aging. Its earliest appearance happened at the age of 30 months under the impact of collagen, a bit later – with ADP and ristomicin, and still later – with H_2O_2 and thrombin. The latest AP in rats at the age of 30 months developed under the impact of adrenaline. Combination of inductors caused their mutual potentiation accelerating AP in aged rats nearly in 2 times (Table).

Quantitative content of discoid platelets in blood of aged rats lowered with aging and was the least one at the age of 30 months (16.5% less than in control). At the same time, the sum of platelets' active forms in observed rats gradually rose with aging and reached maximum values to the age of 30 months. The quantity of disco-echinocytes in their number rose till 40.0% while the number of spherocytes, sphero-echinocytes and bipolar forms reached 59.2%, 96.0% and 3.0 times, respectively. The content of small and large aggregates in bloodstream of the observed aged animals gradually rose by 30 months in 2.2 and 4.7 times, respectively. The content of platelet aggregation in rats between 18-30 months of life increased on 39.0%, exceeding the control level by the end of the observation on 44.7% (Table).

There is no doubt that morphology and functions of a body providing its vital capacity, depend on its genetic program [9,10] and various factors [11,12]. Haemostatic and rheological blood properties [13,14] are among those factors [15,16]. They mostly determine the volume of nutrients' and oxygen intake to tissues [17,18]. They inevitably change in the course of ontogenesis under the influence of many internal and external impacts [19,20]. A significant role in the dynamics of microcirculation is played by

Table 1. Platelet activity in aged rats

Consider indicators	Aged rats, n=95, M±m			control, n=27, M±m
	18 months, n=32	24 months, n=29	30 months, n=34	
AHP, D_{233}/1 ml	1.56±0.024	1.87±0.036*	1.87±0.058**	1.44±0.007
TBA-active products, umol/l	3.60±0.016	3.91±0.023*	4.28±0.032**	3.46±0.016
AOA, %	32.7±0.32	29.2±0.27*	26.4±0.29**	34.8±0.010
Aggregation of platelets with ADP, s	38.4±0.12	35.0±0.14*	33.1±0.16**	39.4±0.07
Aggregation of platelets with collagen,s	31.8±0.09	29.6±0.12*	26.3±0.10**	32.2±0.06
Aggregation of platelets with thrombin,s	51.3±0.14	48.6±0.09*	45.7±0.14**	54.6±0.09
Aggregation of platelets with ristomicin, s	46.1±0.09	43.0±0.13*	39.7±0.17**	47.6±0.10
Aggregation of platelets with H_2O_2, s	40.1±0.14	37.6±0.08*	33.2±0.15**	42.4±0.08
Aggregation of platelets with epinephrine, s	93.4±0.16	88.2±0.17**	79.1±0.24**	98.5±0.07
Aggregation of platelets with ADP and epinephrine, s	35.2±0.08	32.6±0.09*	29.3±0.12**	37.6±0.04
platelet aggregation with ADP and collagen, s	27.6±0.12	25.2±0.16*	22.8±0.19**	28.4±0.08
Aggregation of platelets with epinephrine and collagen, s	31.3±0.07	29.1±0.10*	26.0±0.14**	32.6±0.10
Platelets-discocytes, %	77.2±0.15	72.4±0.19**	68.3±0.25**	79.6±0.15
disco-echinocytes, %	14.0±0.18	15.4±0.16*	17.5±0.17**	12.5±0.07
spherocytes, %	5.1±0.12	7.0±0.14**	7.8±0.12**	4.9±0.05
sphere-echinocytes, %	2.7±0.08	4.0±0.07**	4.9±0.05**	2.5±0.02
bipolar forms, %	0.6±0.02	1.2±0.04*	1.5±0.06**	0.5±0.04
Sum of platelets' active forms, %	22.8±0.19	27.6±0.17**	31.7±0.24**	20.4±0.10
The number of platelets in aggregates, %	4.9±0.05	5.9±0.09**	6.8±0.06**	4.7±0.03
Number of little aggregates (in 100 free platelets)	3.5±0.09	5.7±0.10**	7.2±0.13**	3.3±0.02
Number of medium and large aggregates (in 100 free platelets)	0.14±0.004	0.38±0.003**	0.56±0.007**	0.12±0.005

Conventional signs: reliability of indices' differences in control and aged rats
- <0.05;**-p<0.01*

platelets' activity which is under serious impact of vascular wall and LPO processes in their membranes and in blood plasma [21].

LPO intensification in aged rats was accompanied by the rise of platelets' activity in them which was maximally evident at the age of 30 months. It was obviously connected with sensitivity growth of platelets' receptors to plasma impacts on platelets. The rise of von Willebrand's Factor concentration in blood also refers to them. It is a co-factor of platelets' adhesion. It is apparently accompanied by the rise of receptors' number to it – (GPI b) on the surface of platelets [2]. Receptor rearrangements on platelets' membranes were conditioned by the reaction of hemostasis system on functional state's dynamics of the whole body. They were undoubtedly the consequence of complicated adaptable reactions and membrane composition of platelets which finally conditioned their adaptation to existing age-specific changes in tissues and internals.

AP registration with some inductors in rats between 18-30 months of life confirmed age-dependent gradual strengthening of platelets' aggregative function. At the same time, AP acceleration at binding, including strong agonists of aggregation – collagen and thrombin with receptors on platelets' membrane, could be mostly conditioned by activation of phospholipase C stimulating phosphoinositol way through diacylglycerol, proteinkinase C and phospholyration of the contractile system's proteins. Developing in this case rising quantity of inositol triphosphate promoted more active Ca^{2+} yield out of intraplatelet depot [11]. Strengthening of these mechanisms conditioned evident intensification of actomyosin reduction. It's possible that a great role in rats' AP acceleration while aging was also played by gradual activity strengthening of platelets' enzymatic systems including thromboxane formation causing earlier reaction of platelets on a stimulus [12]. Found age-specific AP acceleration with weak agonists of platelets' aggregation (ADP and adrenaline) has in its basis the development of receptors' surplus quantity on their membranes to ADP, adrenaline and fibrinogen (GPII b-IIIa). It strengthens the activity of phospholipase A_2 leading to age-specific yield of arachidonic acid out of phospholipids and additionally promoting some strengthening of thromboxane A_2 formation [13,22].

Estimation of AP with application of two inductors showed their mutual potentiating action. It confirmed the regularities found at AP research with isolated agonists.

Found IAP increase in aged rats indirectly pointed at the level rise of aggregation inductors (thrombin, ADP, adrenaline) in their blood with aging and at the growth of platelets' basal sensitivity to them [23]. At the same time, the rats at the late stages of ontogenesis were noted to have lowering quantity of intact discoid platelets' forms in their bloodstream. It also pointed at activity increase of their receptors. Number growth of disco-echinocytes and other platelets' active forms in animals' blood with aging coincided with the rise of platelets' aggregative activity. It was found in vitro and was evidently connected, first of all, with expression strengthening of fibrinogenic receptors (GP IIb-IIIa) [24] on their membranes while aging.

4. CONCLUSION

The process of aging touches many parameters of a body, inevitably influencing blood indices. Hemostasis activity is very significant for the process of aging. An important role is played by platelets in this process. Dynamics' determination of platelets' activity in the process of rats' aging can be helpful in opening of age-specific thrombophilia causes. It can also serve the foundation for future experimental researches on prolongation of mammals' lives. We found out in our study that healthy rats between 18 - 30 months of life had gradual rise of platelets' aggregative activity and content increase of platelets' active forms in their blood. It is inevitably accompanied by number increase of freely circulating aggregates of different sizes in rats' blood. Strengthening of platelet activity in aged rats contributes significantly to rising with aging morbid aggravation as it increases sensitivity of a body to negative impacts of environmental factors and promotes the realization of hereditary predisposition to various pathology.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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