

Platelet-Activity Dependence on the Age of Rats with Experimental Dyslipidemia

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Abstract

The purpose of the paper is to reveal the dependence of platelet activity level in rats on the age of their experimental dyslipidemia. The study included 105 healthy male rats (34 rats aged 12 months, 32 rats aged 18 months, and 39 rats aged 24 months) with modeled dyslipidemia. The control group included 91 healthy male rats (30 animals aged 12 months, 32 rats aged 18 months, and 29 animals aged 24 months) under standard vivarium conditions. Biochemical, hematological, and statistical methods were applied. Gradual increasing of functional platelet activity was noticed in the control rats with increasing age. Alimentary modeling of dyslipidemia in the rats resulted in marked platelet-activity increase, growing with the increase in the experimental animals' age. Dyslipidemia modeling in the rats of different age showed the weakening of plasma antioxidant protection and increase in plasma LPO, deepening with the increase in the studied animals' age. The developing disorders in the experimental rats exceeded the age-related changes of platelet activity in the control.

Keywords: Platelets; Aggregation; Aging; Rats; Experimental dyslipidemia

Introduction

Often the pathology develops at the second half of the ontogenesis in mammals and humans [1,2], with an evident, genetically determined base [3-5], ultimately leading to the organism's death [6]. Among other reasons, age-related changes can negatively affect rheological properties of blood cells [7,8] and activity of the hemostasis system [9], often resulting in a thrombophilic situation in their body [8,10].

Currently, physiology and medicine pay great attention to the study of the early stages of the development of different pathologies and their initial mechanisms [11-13]. The researchers are interested in the functional and rheological characteristics of the different blood cells [14-16]. They are highlighted to be very important in the hemostasis system functioning in different pathologies [17-20], including in cardiovascular [21-23] and metabolic diseases [4,24], considered to be very common these days. Atherosclerosis is known to be at the top of the list worldwide, resulting in wide disablement of population and significantly increasing mortality rates in the working-age population [25]. High-platelet activity [26,27] and poor vascular control over them [28,29] were observed at the comprehensive clinical picture of metabolism disorders, leading to the progress of atherosclerosis. This situation significantly activates hemostasis [30] and reduces the efficiency of microcirculation and intensity of metabolism in all tissues [21,31]. At the same time, the state of hemostatic characteristics of platelets at early stages of dyslipidemia development, leading to atherosclerosis, is still studied quite insufficiently.

Considering that the surface with activated platelets is a "buffer state" for all hemostasis processes [32], capable of causing occlusion of different vessels [33,34] under poor disaggregating mechanisms,

experimental works on the detailed study of the dyslipidemia impact on platelet activity are considered to be of great practical interest. It is hard to trace the earliest stages of hemostatic disorders of platelets in humans because very seldom do doctors observe people with the first signs of dyslipidemia. This makes experimental researches in laboratory on animals urgent, with dyslipidemia modeling in them at different stages of the second half of ontogenesis.

The purpose of the paper is to find out the dependence of platelet activity in rats on the age of their experimental dyslipidemia.

Materials and Methods

The study included 105 healthy experimental male rats (34 rats aged 12 months, 32 rats aged 18 months, and 39 rats aged 24 months), with dyslipidemia formed by the alimentary method. The rats were placed in close cells by one specimen for 30 days and fed a high-calorie diet consisting of combined feed (47%), sweet condensed milk (44%), vegetable oil (8%), and vegetable starch (1%), which provided the following diet: 29.6% fats, 14.8% proteins, and 55.6% carbohydrates [35].

The control group included 91 healthy male rats, including 30 animals aged 12 months, 32 rats aged 18 months, and 29 animals aged 24 months. They received combined feed manufactured by Laboratorkorm, Russia, in full and were not affected. All animals were healthy throughout the preobservation period and did not participate in any experiments before.

Concentration of total cholesterol (TC) and triglycerides (TG) was determined by the enzymatic colorimetric method using "Vital Diagnosticum" kit. High-density lipoprotein (HDL) cholesterol level in plasma was found out by the enzymatic colorimetric method using "Olvex Diagnosticum" kit. Concentration of low-density lipoprotein (LDL) cholesterol was determined by calculation. Concentration of

very low-density lipoprotein (VLDL) cholesterol was calculated using the formula: $VLDL\ TC = TG / 2.2$.

Intensity of plasma lipid peroxidation (LPO) was assessed by the content of thiobarbituric acid (TBA)-active compounds using "Agat-Med" kit and of acylhydroperoxide (AHP), taking into account the plasma-antioxidant activity (AOA) [36]. The number of platelets in the capillary blood was determined in Goryaev chamber. Platelet aggregation was determined by the visual micro method [37] using the following as inducers: ADP ($0.5 \cdot 10^{24}M$); collagen (dilution of main suspension 1:2); thrombin (0.125 u/ml); ristomycin (0.8 mg/ml); epinephrine ($5 \cdot 10^{26}M$); hydrogen peroxide ($7.3 \cdot 10^{23}M$); and a combination of ADP and epinephrine, ADP and collagen, epinephrine and collagen. Considered values were evaluated once in both groups of animals. The results were statistically processed by Student's t-test.

Results

Observed that experimental and control rats of all ages had not differed by all considered indicators before the study. With increasing age, reliable increase in the number of plasma AHP and TBA compounds was observed in the control group while its antioxidant activity was lowered. Dyslipidemia formation in the experimental rats was accompanied with its greater expression, increased plasma LPO activity, and decreased antioxidant protection. At the same time with increasing age of the animals, these changes deepen, exceeding the severity of age-related changes in the control. LPO level was the highest at dyslipidemia formation in 24-month-olds: AHP, $2.46 \pm 0.018 D_{233}/1\text{ mL}$; TBA-active compounds, $5.38 \pm 0.026\text{ mcmol/L}$; and the lowest AOA $20.1 \pm 0.32\%$. In the control 24-month-old rats, these values made $1.95 \pm 0.033 D_{233}/1\text{ mL}$, $4.22 \pm 0.042\text{ mcmol/L}$, and $26.2 \pm 0.27\%$, respectively (Table 1).

The platelet concentration at the age of 12 months was similar in the experimental and control rats. In the control group with increasing

age, platelet aggregation was noted to be increased in response to all agonists and their combinations. The most active platelet aggregation developed in these animals under collagen; platelet aggregation under ristomycin, H_2O_2 , and ADP was observed later, and afterward—under thrombin. The latest platelet aggregation in the control rats of all ages was noticed under epinephrine. Combinations of inducers provide interpotentiation and accelerate platelet aggregation, resulting in its faster appearance, twice as fast compared to the individual inducers (Table 1).

Progressive acceleration of platelet aggregation, associated with increasing age at dyslipidemia formation, was observed in the experimental animals. The greater the studied animal's age, the more actively its platelets reacted to all the inducers and their combinations, that significantly exceeded the age-related changes in the control. Thus, the platelets in the animals with developed dyslipidemia at all ages most actively responded to collagen and ADP, poorly to H_2O_2 and ristomycin, and even less to thrombin and epinephrine. Duration of platelet aggregation in the rats with alimentary dyslipidemia in response to the combination of inducers also reliably increased the control, incrementing with increasing age of the animals.

Discussion

Hypercholesterolemia and hypertriglyceridemia, common for humans [38], are noticed in the rats at dyslipidemia modeling, as well as the weakening of the plasma antioxidant capacity, accompanied by the gradual increase of AHP and TBA-active compounds and inevitably worsened metabolism in the tissues. In addition, plasma LPO activation caused alteration of the surface structures of blood cells [39], including their most significant hemostatic population—platelets—that quite negatively affected their functions [40].

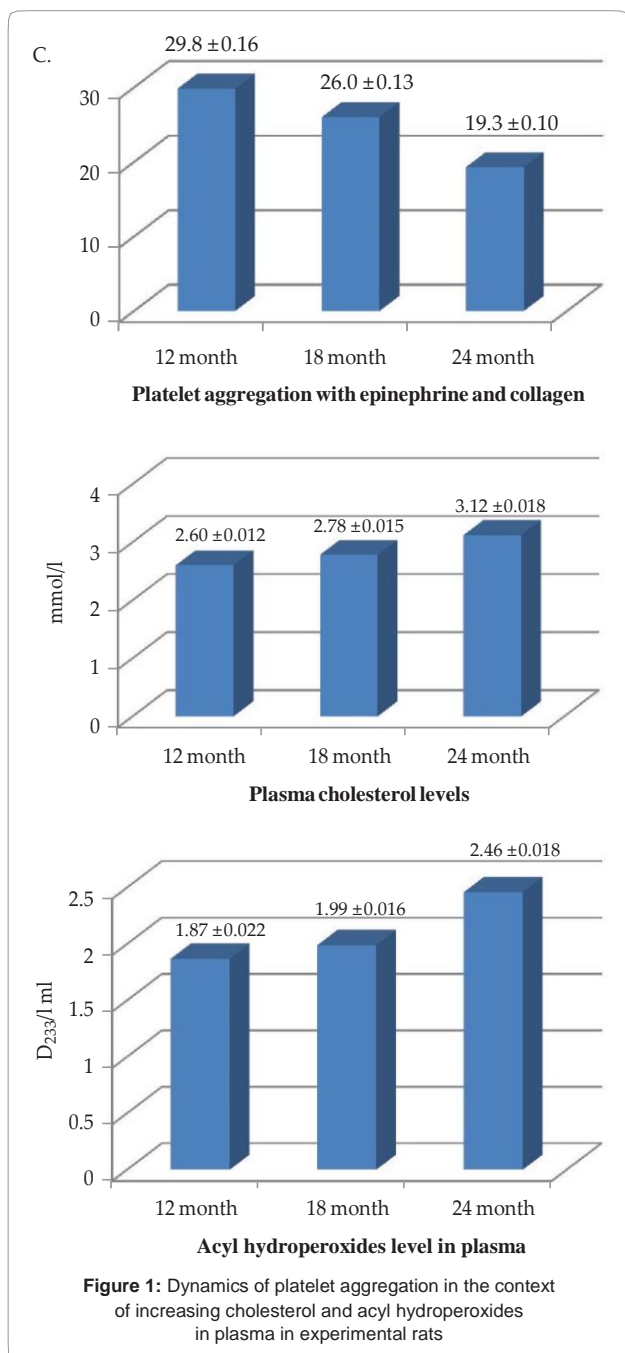
Plasma-lipid composition changes, formed in the modeling, affected the ratio between platelet membrane lipids, activating lipid

Indicators	Experimental group, M \pm m, n \square 105			The control group, M \pm m, n \square 91		
	12 months, n \square 34	18 months, n \square 32	24 months, n \square 39	12 months, n \square 30	18 months, n \square 32	24 months, n \square 29
Total cholesterol, mmol/l	2.60 \pm 0.012	2.78 \pm 0.015	3.12 \pm 0.018	2.18 \pm 0.009*	2.22 \pm 0.012**	2.25 \pm 0.010**
HDL cholesterol, mmol/l	1.00 \pm 0.010	0.95 \pm 0.009	0.91 \pm 0.008	1.12 \pm 0.008*	1.11 \pm 0.015**	1.09 \pm 0.008**
LDL cholesterol, mmol/l	0.89 \pm 0.015	1.07 \pm 0.016	1.41 \pm 0.018	0.60 \pm 0.008*	0.64 \pm 0.007**	0.68 \pm 0.005**
VLDL, mmol/l	0.71 \pm 0.014	0.76 \pm 0.009	0.80 \pm 0.012	0.46 \pm 0.007*	0.47 \pm 0.003**	0.48 \pm 0.006**
TG, mmol/l	1.56 \pm 0.010	1.68 \pm 0.014	1.76 \pm 0.011	1.02 \pm 0.004*	1.03 \pm 0.006**	1.05 \pm 0.007**
AHP, $D_{233}/1\text{ ml}$	1.87 \pm 0.022	1.99 \pm 0.016	2.46 \pm 0.018	1.52 \pm 0.018*	1.60 \pm 0.024**	1.95 \pm 0.033**
TBA compounds mcmol/l	3.91 \pm 0.019	4.78 \pm 0.020	5.38 \pm 0.026	3.61 \pm 0.022*	3.80 \pm 0.016**	4.22 \pm 0.042**
AOA%	30.3 \pm 0.37	27.0 \pm 0.28	20.1 \pm 0.32	32.6 \pm 0.24*	30.7 \pm 0.32**	26.2 \pm 0.27**
AT with ADP, C	36.2 \pm 0.12	32.4 \pm 0.09	29.2 \pm 0.10	39.2 \pm 0.08*	38.4 \pm 0.12**	35.0 \pm 0.14**
AT with collagen, C	30.8 \pm 0.16	26.6 \pm 0.09	21.9 \pm 0.14	32.0 \pm 0.14*	31.8 \pm 0.09**	29.6 \pm 0.12**
AT with thrombin, C	51.6 \pm 0.13	43.6 \pm 0.14	38.4 \pm 0.12	54.5 \pm 0.16*	51.3 \pm 0.14**	48.6 \pm 0.09**
AT with ristomycin, C	45.2 \pm 0.18	41.3 \pm 0.16	34.7 \pm 0.15	47.3 \pm 0.13*	46.1 \pm 0.09**	43.0 \pm 0.13**
AT with H_2O_2 , C	40.1 \pm 0.10	36.3 \pm 0.18	30.8 \pm 0.16	42.1 \pm 0.10*	41.1 \pm 0.14**	37.6 \pm 0.08**
AT with epinephrine, C	96.3 \pm 0.26	83.0 \pm 0.28	75.7 \pm 0.35	98.1 \pm 0.22*	93.4 \pm 0.16**	88.2 \pm 0.17**
AT with ADP and epinephrine, C	34.0 \pm 0.14	30.2 \pm 0.12	24.1 \pm 0.09	37.3 \pm 0.13*	35.2 \pm 0.08**	32.6 \pm 0.09**
AT with ADP and collagen, C	25.4 \pm 0.11	22.3 \pm 0.07	19.2 \pm 0.08	28.5 \pm 0.09*	27.6 \pm 0.12**	25.2 \pm 0.16**
AT with epinephrine and collagen, C	29.8 \pm 0.16	26.0 \pm 0.13	19.3 \pm 0.10	32.3 \pm 0.11*	31.3 \pm 0.07**	29.1 \pm 0.10**

Note: Significant differences between the experimental and control rats within their age group:

* $p < 0.05$; ** $p < 0.01$.

Table 1: Biochemical and hematological indicators in two-year-old rats against the background of dyslipidemia modeling



peroxidation in them. It quickly disturbed receptor and postreceptor mechanisms of platelet functioning in the model rats. The resulting lipid imbalance in the body also led to negative dynamics in the regulation of platelets of ion and antioxidant status, which ensured negative changes in their metabolism and structural and functional properties in the vessels [41].

Increased platelet sensitivity to aggregation inducers in the experimental rats was provided through the activation of certain mechanisms. So, density of glycoproteins Ia-IIa and VI, involved into blood-platelet adhesion, gradually increased on the surface of their platelets, as can be seen at PA intensification in response to

collagen [32,42]. Increased platelet adhesion in the experimental rats should be associated with overexpression of the receptors to von Willebrand factor on their surface. This mechanism of increasing of platelet-adhesive activity in these rats was recorded by intensification of platelet aggregation with ristomycin affecting platelets equally to the subendothelial vascular structures. Given that the ristomycin platelet aggregation needs von Willebrand factor fixing by one side of the molecule to ristomycin (like to collagen), and by the other — to platelets via their receptor — Ib, strengthening of “adhesion axis” formation could be confirmed in the experimental rats: ristomycin (collagen)—von Willebrand factor—GPIb [43]. Thus, significant increase in the number of sites of vWF binding to platelet membranes in the experimental rats is an important mechanism to provide their excessive platelet adhesion [9,41].

Increased platelet sensitivity to collagen in the experimental rats with age should be associated with the increased number of receptors on the platelet surface to it, potentiated by age-related changes and especially by dyslipidemia [24,26]. This is inevitably accompanied by activation of phospholipase C, stimulation of diacylglycerol synthesis and protein kinase C, followed by marked phosphorylation of proteins of the contractile system [29]. Under these conditions, inositol triphosphate actively stimulates Ca²⁺ flow from the platelet pool, helping quickly reduce actomyosin that is most evident at the oldest age.

An ADP inducer, related to weak inducers of platelet aggregation [25], more actively stimulated platelets during the modeling. This was caused by its active interaction with a growing number of receptors on the platelet membranes in the experimental rats with increasing age. It resulted in fibrinogen-receptor expression on the platelet surface, gradually increased with age, with activation of phospholipase A₂, providing excision of arachidonic acid from membrane phospholipids [1,22] (Figure 1).

The revealed increase with age of platelet aggregation in response to the combination of inducers in the experimental rats indicates indirectly an increase of an inducer aggregation level (thrombin, ADP, epinephrine) in their blood at dyslipidemia formation, reliably higher than this dynamic in the control. Increasing of receptor activity in the experimental rats with age should be considered to be higher than in the control [44]. Their increased ability in blood to aggregation against the background of dyslipidemia modeling is also associated with increased age-related changes against the background of overexpression of fibrinogen receptors (GP IIb-IIIa) on their membranes.

Conclusion

Dyslipidemia modeling in rats of different ages showed the weakening of plasma antioxidant protection and increase in plasma LPO, deepened with increasing age of the studied animals. The developing disorders in the experimental rats exceeded the age-related changes of platelet activity in the control. It is clear that with age increasing, it is necessary to strictly control the blood-lipid level and to avoid dyslipidemic situations in the body, more activating platelets, and increasing risk of thrombophilia over the years.

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