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Physiological Features Of Platelet Aggregation In Newborn Piglets.

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ABSTRACT

A very physiologically important component of the homeostasis of the body is thrombocytic hemostasis, whose activity largely determines the fluid properties of the blood and thus the rate of development of the animal during early ontogeny. All this is true for the phase of newborn. At present, there is an opinion that the level of functional activity of thrombocytic hemostasis is largely related to the success of the formation of the body's structures, the formation of their functional activity. In this connection, studies of various aspects of platelet activity in piglets of mollusc nutrition are very relevant. As a result of the study, piglets of mollusc nutrition showed an increase in the adhesive and aggregation ability of the blood platelets. The found regularity is based on an increase in the activity of receptor and postreceptor mechanisms of platelets. This is true with regard to the effect of the strong and weak aggregation inducers on the process of platelet aggregation in vitro and in vivo in piglets. A very important mechanism of increasing platelet activity in piglets during the neonatal phase is the tendency to intensify the metabolism of arachidonic acid in their platelets by cyclooxygenase and thromboxane synthetase and activation of the secretion of adenosine diphosphate from them, with a tendency to increase its content in the granules of platelets. The increase in hemostatic activity of platelets ensures adaptation of their microcirculation to the current conditions of life, creating the best conditions for trophic tissue.

Keywords: piglets, newborn phase, platelets, aggregation, adhesion, secretion.

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INTRODUCTION

The hemostatic system is physiologically important and very complex [1,2]. Its successful operation ensures the preservation of blood in the liquid state in the lumen of the vessel [3,4] and the rapid local course of the formation of thrombus in the event of damage to the vessel wall [5,6,7]. A clear work of hemostasis ensures minimization of blood loss and preservation of the organism's viability [8,9]. Taking into account that hemostasis influences fluidity of blood along the vessels [10,11], its great importance for tissue trophism and metabolic processes in tissues [12,13,14], including pigs becomes clear.

Previous studies have shown that the optimal activity of hemostasis provides the necessary level of viability of animals and their development processes [15, 16]. It becomes clear that without a detailed study of many aspects of physiology, including hemostasis, further intensification of pig production is impossible, since hemostasis is not only a system that supports the body, but also an important "point" of potential impact for the regulation of the functional state of the organism in the development of dysfunctions in the background adverse environmental conditions [17,18,19].

A very physiologically important component of homeostasis of the body is thrombocytic hemostasis, whose activity largely determines the fluid properties of the blood and thus the rate of metabolic processes in the body [20,21]. All this is true for the phase of newborn. At present, there is an opinion that the level of functional activity of platelet hemostasis is largely related to the success of the formation of body structures, the formation of their functional activity [22,23]. In this connection, studies of various aspects of platelet activity in newborn animals, including piglets, are important for maintaining their homeostasis at the very beginning of their growth and development [24].

Taking into account all the above, the goal was formulated: to study the physiological features of platelet activity in healthy piglets during the phase of colostrum nutrition.

MATERIALS AND METHODS

The research was conducted in strict accordance with ethical principles established by the European Convent on protection of the vertebrata used for experimental and other scientific purposes (adopted in Strasbourg in March 18, 1986, and confirmed in Strasbourg in June 15, 2006) and approved by the local ethic committee of Federal State Budgetary Educational Institution of Higher Education “Vologda State Dairy Farming Academy by N.V. Vereshchagin” (Record №12 dated December 3, 2015), the local ethic committee of All-Russian SII of Physiology, Biochemistry and Animals' feeding (Record №11, dated December 4, 2015) and the local ethic committee of Russian State Social University (Record №16, dated December 7, 2015).

The study was performed on 38 healthy pigs of large white breed, which were inspected and examined for the phase of newborns 5 times: for 1 day, for 2 days, for 3 days, for 4 days and 5 days of life. All piglets were obtained from healthy sows with 2-3 farrowing.

In all piglets, platelet aggregation activity (AP) was determined by visual micromethod with the use of thrombin (0.125 U/ml), ADP (0.5×10^{-4} M), H_2O_2 (7.3×10^{-3} M), collagen (dilution 1: 2 main suspension), ristomycin (0.8 mg/ml), adrenaline (5.0×10^{-6} M) in plasma, standardized by the number of platelets to $200x10^9$ platelets [25].

In the thrombocytes of the examined pigs, the intensity of the exchange of endogenous arachidonic acid was determined indirectly, taking into account the enzymatic activity of cyclooxygenase and thromboxane synthetase, indirectly recorded with three transfer probes on a photoelectrocolorimeter [26]. Also in platelets of animals, ADP and the intensity of its secretion against the background of thrombin thrombocyte stimulation were evaluated [26].

Intravascular aggregation of thrombocytes was elucidated using a phase contrast microscope [27]. The results of the study were processed using the Student's test.

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RESULTS

All the examined newborn piglets showed a normal number of platelets in the blood. At the same time, for them on the 1st day of life the time of onset of AP in response to collagen was 36.1±0.09 s. It decreased gradually to 33.1±0.08 s by the end of the neonatal phase (Table 1). A similar acceleration of AP in newborn piglets was observed under the action of ADP - by 8.6%, H_2O_2 by 8.8% and ristomycin by 7.6%. Somewhat later, thrombin AP developed (by the end of the phase in 55.0±0.10 s) and adrenaline AP (by the end of the phase in 98.5±0.18 s).

In the blood of piglets of mammary nutrition, the levels of free circulating small and large platelet aggregates gradually increased, amounting to 3.4±0.05 per 100 free-standing platelets and 0.15±0.08 per 100 free-standing platelets on day 5 of life. At the same time, the number of platelets entering the aggregation process in piglets increased by 13.8% at the end of the observation, which emphasized the increased platelet aggregation in vivo at this stage of their development.

An important mechanism that increases the functional activity of platelets in piglets in the first 5 days of life can be considered amplification in platelets of the intensity of the metabolism of arachidonic acid with activation of thromboxane formation. This was indirectly indicated by the increase in AP in a simple transfer sample (from 33.9±0.06% to 38.7±0.04%). The intensified metabolism of arachidonic acid in the blood plates of pigs was found to be possible due to the revealed activation of both enzymes of its transformation in platelets - cyclooxygenase and thromboxane synthetase. The severity of the recovery of AP in the collagen-aspirin test, which indirectly estimates the activity of cyclooxygenase in platelets, increased over the considered age from 64.9±0.09% to 69.2±0.06%. The activity of AP recovery in the collagen-imidazole sample, which allows to indirectly evaluate the functional activity of thromboxane synthetase in blood plates, also increased in piglets during the course of observation from 55.1± 0.08% to 59.5±0.09%.

Another important mechanism for enhancing the functional activity of platelets in piglets during the neonatal phase can be considered as the tendency in the study to increase the content of ADP (by 3.2%) and the activity of its secretion (by 12.8%).

DISCUSSION

The existing body of knowledge on the physiology of piglets is still not complete [28,29]. In this regard, the need for modern practical biology in the detailed study of vital systems in the pig's body is becoming increasingly acute [30]. Among these systems, able to bind the body together at any age, is a hemostatic system in which platelets play an important role [31]. The level of their activity at any age seriously determines the rheology of blood in the microcirculatory bed and thus the activity of metabolism in tissues [32,33]. Despite the importance of platelet hemostasis activity and the fine mechanisms providing it, their condition in healthy piglets during the neonatal phase remains insufficiently studied [34].

Based on the facts obtained in the study, it can be said that in healthy piglets of mammary nutrition, the adhesive ability of blood plates tends to increase due to a simultaneous increase in the concentration in their blood of vWF (FW) factor, which is a cofactor of platelet adhesion and an increase in the number of receptors to it - (GPI c) on the surface membranes of the blood platelets [35]. The activation of these mechanisms in the examined pigs could be judged by the acceleration of aggregation of their platelets in response to ristomycin, which, in its ability to influence platelets, is similar to subendothelial vascular structures [36]. It is precisely established that FW is connected by one end of the molecule with collagen, and the other with a platelet through the platelet receptor-glycoprotein Ib, forming a morphological basis of adhesion [37]. It is represented by a chain: collagen - FW - GPIb. In this connection, the acceleration of AP with ristomycin suggests the development in piglets at the very onset of their ontogenesis of an increase in the number of these receptors on the platelet membranes. The found acceleration of AP in response to the rest of the inducers also showed an increase in the number of receptors on the surface of the blood platelets from 1 to 5 days in piglets.

Evaluation of the influence of strong and weak aggregation inducers on the process of platelet aggregation in vitro in the examined pigs made it possible to reveal the features of their influence on platelets

on the physiological pathways of their activation. This made it possible to evaluate the course of AP in various ways, activation of which is typical for normal blood flow conditions [38].

The increase in the number of platelet aggregates in the blood of newborn piglets was caused by changes in receptor and postreceptor mechanisms in platelets [39]. This was due to a slight increase in hemostatic activity of platelets with ineffective adhesion and in vivo aggregation. This circumstance should be associated with the growth of expression on their membrane of receptors of different species and especially fibrinogen receptors (GP IIb-IIIa). The development in these conditions of stimulation of the catalytic properties of the phospholipids of the plasma membrane ensures an increase in the generation of factor XA and thrombin on it [40,41,42].

A very significant intra-platelet mechanism for increasing platelet activity in piglets during the neonatal phase can be considered a small intensification in their metabolism of arachidonic acid due to a gradual increase in the activity of platelet cyclooxygenase and thromboxane synthetase. In addition, in platelet granules, ADP accumulated more strongly with a tendency to increase its secretion from them.

CONCLUSION

The increase in the number of platelet aggregates in the blood of newborn piglets was caused by changes in receptor and post-receptor mechanisms in platelets. This was due to an increase in their haemostatic activity with an undetectable increase in adhesion and aggregation. The revealed tendency to the growth of platelet activity in piglets during the phase of newborn infants determines the optimal level of microcirculation in tissues in them, which is adequate, on the one hand, to their genetic program, and on the other hand, to the influences of the environment, and can be considered a specific adaptive reaction affecting their growth and development.

Table 1. Platelet activity indices in newborn piglets

Indicators	Newborn phase, n=38, M±m				
	1 day of life	2 day of life	3 day of life	4 day of life	5 day of life
Aggregation of platelets with ADP, s	45.6±0.12	45.0±0.14	44.2±0.16	43.3±0.09 p<0.05	42.0±0.12 p<0.05
Aggregation of platelets with collagen, s	36.1±0.09	35.2±0.08	34.2±0.11	33.1±0.08 p<0.05	32.2±0.10 p<0.05
Aggregation of thrombocytes with thrombin, s	60.2±0.07	58.4±0.09	56.3±0.08 p<0.05	55.0±0.10 p<0.05	54.1±0.06 p<0.01
Aggregation of platelets with H_2O_2, s	48.2±0.08	47.8±0.06	46.5±0.09	45.2±0.10 p<0.05	44.3±0.05 p<0.05
Aggregation of platelets with ristomycin, s	47.8±0.12	46.8±0.06	46.0±0.10	45.2±0.07 p<0.05	44.4±0.10 p<0.05
Aggregation of platelets with adrenaline, s	101.5±0.17	100.2±0.21	99.2±0.16	98.5±0.18	97.3±0.14 p<0.05
Recovery of platelet aggregation in a collagen-aspirin test, %	64.9±0.09	65.7±0.08	66.2±0.10	67.8±0.09 p<0.05	69.2±0.06 p<0.05
Restoration of platelet aggregation in a collagen-imidazole sample, %	55.1±0.08	56.2±0.06	56.9±0.07	57.5±0.08 p<0.05	59.5±0.09 p<0.05
Aggregation of platelets in a simple transfer sample, %	33.9±0.06	34.5±0.05	35.7±0.07	36.8±0.08 p<0.05	38.7±0.04 p<0.05
The content of ADP in platelets, mmol/10^9 platelets	3.00±0.09	3.03±0.11	3.05±0.08	3.08±0.10	3.12±0.07

The degree of secretion of ADP from platelets on the background of stimulation, %	31.2±0.32	31.9±0.15	32.6±0.10	33.9±0.12	35.2±0.05 p<0.05
The number of platelets in the aggregates, %	6.5±0.15	6.6±0.10	6.8±0.12	7.1±0.09 p<0.05	7.4±0.09 p<0.01
The number of small aggregates of 2-3 platelets per 100 freely lying platelets	2.8±0.03	2.9±0.05	3.0±0.04	3.2±0.06 p<0.05	3.4±0.05 p<0.01
The number of medium and large aggregates, 4 or more platelets, per 100 free-lying platelets	0.10±0.008	0.11±0.005	0.12±0.006 p<0.05	0.14±0.007 p<0.01	0.15±0.008 p<0.01

Legend: p - reliability of the dynamics of newborns taken into account with respect to the onset of the phase.

REFERENCES

[1] Mischenko VP, Mishchenko IV. (2003) Physiology of the hemostasis system. Poltava, 124.

[2] Medvedev IN, Danilenko OA. (2010) Effectiveness of vascular wall activity correction in patients with arterial hypertension, metabolic syndrome, and oculo-vascular occlusion. Russian Journal of Cardiology. 3:64-67.

[3] Medvedev IN. (2018) Activity of Platelets' Aggregation in Patients of The Second Mature Age with Arterial Hypertension of The 3rd degree. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(1):651-656.

[4] Medvedev IN. (2018) Aggregation of Thrombocytes in People of Second Adulthood with Arterial Hypertension of the 2 Rd Degree. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(1):870-875.

[5] Medvedev IN, Danilenko OA. (2010) Comparative effects of therapeutic complexes on vascular wall activity in patients with arterial hypertension, metabolic syndrome, and recent ocular vessel occlusion. Cardiovascular therapy and prevention. 9(7):27-32.

[6] Bikbulatova AA. (2018) The Impact Of Medicinal-Prophylactic Trousers' Daily Wearing On Pregnancy Course In The Third Term Of Women With Habitual Miscarriage Of Fetus. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(3) : 663-671.

[7] Bikbulatova AA, Andreeva EG, Medvedev IN. (2018) Hematological Features Of Patients With Osteochondrosis Of The Spine. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(3) : 1089-1095.

[8] Bikbulatova AA. (2018) Formation Of Psychological Comfort In Women With Habitual Miscarriage Of Pregnancy Against The Background Of Their Daily Wearing Of Medicinal Prophylactic Trousers. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(3) :1417-1427.

[9] Medvedev IN. (2018) Disaggregating Vascular Impacts on Platelets in Patients with Arterial Hypertension Of The 3rd Degree. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(1):663-669.

[10] Skripleva EV, Vorobyeva NV, Kiperman YaV, Kotova OV, Zatsepin VI, Ukolova GB. (2018) The Effect Of Metered Exercise On Platelet Activity In Adolescents. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(3) : 1150-1154.

[11] Medvedev IN. (2018) Peculiarities of vascular control over platelet aggregation in patients with Arterial hypertension of the 3rd degree at metabolic syndrome. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(1):258-264.

[12] Medvedev IN. (2018) Functional Peculiarities Of Platelet Activity In Persons With Arterial Hypertension Of The High Degree Developing Against The Background Ofhypodynamia At Metabolic Syndrome. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(1):308-314.

[13] Medvedev IN. (2018) Dis Aggregative Capabilities of Vascular Wall in Respect of Erythrocytes in Patients with Arterial Hypertension and Dislipidemia. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(1):811-816.

[14] Medvedev IN. (2018) Disaggregative Impacts of Vascular Wall on Platelets of Patients with Arterial Hypertension and Dyslipidemia. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(1):857-863.
[15] Medvedev IN. (2018) Vascular Disaggregative Control Over Neutrophils In Patients With Arterial Hypertension And Dyslipidemia. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(1):864-869.
[16] Medvedev IN. (2018) Development of Platelet Dysfunctions at Arterial Hypertension with Dyslipidemia. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(1): 439-444.
[17] Medvedev IN. (2018) Physiological Reaction of Erythrocytes' Microrheological Properties in Persons of The Second Mature Age on Prolonged Hypodynamia. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(1):577-582.
[18] Medvedev IN. (2018) Physiological Peculiarities of Erythrocytes' Aggregation in Rats Of Elder Ages. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(1):716-721.
[19] Medvedev IN. (2018) Physiological Dynamics of Erythrocytes' Cytoarchitecture In Aged Rats. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(1):736-740.
[20] Medvedev IN, Gamolina OV. (2008) Lisinopril effects on platelet activity in patients with arterial hypertension and impaired glucose tolerance. Russian Journal of Cardiology. 3:45-48.
[21] [21] Medvedev IN, Kumova TA. (2007) Angiotensin II receptor inhibitors: role and place in arterial hypertension and metabolic syndrome treatment. Russian Journal of Cardiology. 5 : 97-99.
[22] Medvedev IN, Gromnatskii NI, Golikov BM, Al'- Zuraiki EM, Li VI. (2004) Effects of lisinopril on platelet aggregation in patients with arterial hypertension with metabolic syndrome. Kardiologiia. 44(10):57-59.
[23] Medvedev IN, Gromnatskii NI, Mokhamed A.-ZE.(2004) Comparative Assessment of Effects of Qadropril and Enalapril on Intravascular Activity of Platelets in Hypertensive Patients With Metabolic Syndrome. Kardiologiia. 44(12):44-46.
[24] Medvedev IN, Gromnatskii NI. (2005) Normodipin in correction of platelet rheology in hypertensive patients with metabolic syndrome. Terapevticheskii Arkhiv. 77(6):65-68.
[25] Shitikova AS. (1999) Visual micromethod of the study of platelet aggregation. In the book Hemostasis. Physiological mechanisms, principles of diagnosis of the main forms of hemorrhagic diseases. St. Petersburg, 49-53.
[26] Ermolaeva TA, Golovina OG, Morozova T.V. (1992) Program of clinical and laboratory examination of patients with thrombocytopathy. St. Petersburg, 25.
[27] Shitikova AS, Tarkovskaya LR, Kargin V.D. (1997) A method for determining intravascular platelet activation and its significance in clinical practice. Clinical and laboratory diagnostics. 2: 23-35.
[28] Makhov AS, Medvedev IN. (2018) General Assessment of The Health Status of Children with Down Syndrome Who Have Low Physical Activity. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(2) : 427-431.
[29] Makhov AS, Medvedev IN. (2018) Basics of Prevention and Correction of Asthenic Syndrome in Young Footballers With Down Syndrome. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(2) : 522-526.
[30] Makhov AS, Medvedev IN. (2018) Problems of Physical Rehabilitation of Children with Down Syndrome with Injuries of The Lower Limb. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(2) : 615-619.
[31] Makhov AS, Medvedev IN. (2018) Evaluation of the effectiveness of the complex rehabilitation of children with oligophrenia in the degree of imbecility, who underwent fracture of the lower limb. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(2) : 731-736.
[32] Makhov AS, Medvedev IN. (2018) The Ability to Reduce the Severity of Motor Disorders in Children With Cerebral Palsy. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(2) : 991-996.
[33] Makhov AS, Medvedev IN. (2018) General improvement of children with Down syndrome by means of regular soccer lessons. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(2) : 1014-1019.
[34] Makhov AS, Medvedev IN. (2018) Correction Of Asthenia In Football Players With Down Syndrome. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(2) : 1161-1166.
[35] Makhov AS, Medvedev IN. (2018) Functional Features Of Young Football Players With Down Syndrome. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(3) : 678-683.

[36] Makhov AS, Medvedev IN. (2018) Dynamics Of A Functional State Of Players With Oligophrenia In Conditions Of Sports Competitions. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(3) : 938-941.

[37] Makhov AS, Medvedev IN. (2018) Functional Features Of Children With Cerebral Palsy Having Low Physical Activity. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(3) :1428-1432.

[38] Bikbulatova AA, Andreeva EG, Medvedev IN. (2018) Hematological Features Of Patients With Osteochondrosis Of The Spine. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9(3) :1089-1095.

[39] Bikbulatova AA, Karplyuk AA, Parshin GN, Dzhafar-Zade DA, Serebryakov AG. (2018) Technique for Measuring Vocational Interests and Inclinations in High-School Students with Disabilities. Psikhologicheskaya nauka i obrazovanie-psychological science and education. 23(2) : 50-58.doi: 10.17759/pse.2018230206

[40] Apanasyuk LA, Soldatov AA. (2017) Socio-Psychological Conditions for Optimizing Intercultural Interaction in the Educational Space of the University. Scientific Notes of Russian State Social University. 16(5-144) : 143-150. doi: 10.17922/2071-5323- 2017-16-5-143-150.

[41] Maloletko AN, Yudina TN.(2017) (Un)Making Europe: Capitalism, Solidarities, Subjectivities. Contemporary problems of social work. 3 (3-11) : 4-5.

[42] Pozdnyakova ML, Soldatov AA. (2017) The Essential and Forms of the Approaches to Control the Documents Execution. 3 (1-9): 39-46. doi: 10.17922/2412-5466-2017-3-1-39-46.