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Physiological Features Of Primary Hemostasis In Newborns Calves With Functional Digestive Disorders.

Zavalishina S Yu*.

Russian State Social University, st. V. Pika, 4, Moscow, Russia, 129226.

ABSTRACT

Changes in the activity of primary hemostasis in newborn calves with functional disorders of the digestive system are of practical importance. It is clear that the increase in the activity of the primary link of hemostasis plays a leading role in the activation of hemostasis in general, the increase in blood viscosity and the formation of a tendency towards intravascular thrombosis. Disorders of platelet aggregation, antiaggregation activity of the vascular wall and intravascular activity of the platelets in newborn calves with functional disorders of the digestive system required further study. For this reason, the extent of changes in the activity of platelets in newborn calves with functional disorders of the lipid composition of their membranes, the level of peroxidation and antioxidant protection of blood platelets, as well as the state of arachidonic acid exchange was determined. It was found that in newborn calves with functional digestive disorders increases the aggregation function of platelets in vitro and in vivo. They are based on the shifts that develop in these calves in the lipid composition of the platelet membranes, the increase in the average molecules in them, the activation of plasma lipid peroxidation and platelets, a significant increase in vlllebrand factor vascular wall synthesis, the weakening of prostacyclin formation and the intensification of platelet formation. It can be considered that impairments in the functioning of primary hemostasis in calves with functional digestive disorders are an important basis for the impairment of microcirculation and tissue trophism in them.

Keywords: calves, hemostasis, functional digestive disorders, platelets.

**Corresponding author*

INTRODUCTION

Identifying the dynamics of the activity of primary hemostasis in newborn calves with functional digestive disorders is of practical importance [1,2,3], since it is the activation of the primary link of hemostasis that plays a leading role in the activation of hemostasis in general [4-7], increasing viscosity [8,9] and worsening of the blood rheology [10] with a tendency to intravascular thrombosis [10,11]. At the same time, violations of the aggregation ability of platelets, the antiaggregation activity of the vascular wall, and the intravascular activity of the platelets in newborn calves with functional disorders of digestion have been very poorly studied. The degree of change in platelet activity in newborn calves with functional digestive disorders of the lipid composition of their membranes, the level of peroxidation and the antioxidant protection of blood platelets, as well as the level of arachidonic acid exchange in them, has not been determined. There is fragmentary information that functional disorders of the digestive system are accompanied in newborn calves by an increase in the plasma level of medium molecules [12], which can disrupt many functions of the body. The extent of the increase in average molecules in platelets, contributing in many ways to the formation of thrombocytopeny, has not been elucidated.

The aim of the work is to investigate the features of the violation of primary hemostasis in newborn calves with functional digestive disorders.

MATERIALS AND METHODS

Research was conducted in strict accordance with ethical principles established by the European Convention on protection of the vertebrata used for experimental and other scientific purposes (adopted in Strasbourg March 18, 1986, and confirmed in Strasbourg June 15, 2006) and approved by the local ethic committee of Russian State Social University (Record №12 dated December 3, 2015).

The study included 153 newborn calves with functional digestive disorders for a period of 1-3 days from healthy cows 1-2 calves. Feeding and maintenance was carried out in standard calf conditions. The control group consisted of 267 healthy newborn calves. Blood sampling was carried out in the morning. The survey included the following indicators. The level of medium molecules in plasma and washed and resuspended platelets was determined. The activity of plasma lipid peroxidation (LPO) was determined by the content of thiobarbituric acid-active products by the set of Agat-Med, acyl hydroperoxide (AHP), and intra platelet LPO by concentration of the basal level of malonic dialdehyde (MDA) in the reduction of thiobarbituric acid and in the recovery of thiobarbituric acid and by the curve of the benthic acid; The intra-platelet antioxidant system characterized the activity of catalase and superoxide dismutase.

The cholesterol content in washed and resuspended platelets was determined by an enzymatic colorimetric method using Vital Diagnosticum and phospholipids using phosphorus. The activity and time of formation of endogenous thromboplastin were also investigated. For indirect assessment of arachidonic acid metabolism in platelets, as well as the activity of cyclooxygenase and thromboxane synthetase in them, 3 transfer samples with registration of platelet aggregation (AP) on a photoelectric colorimeter were used [6]. The number of platelets in capillary blood in the Goryaev chamber was counted. The aggregation ability of platelets was investigated by a visual micromethod using as inducers ADP (0.5×10^{-4} M), collagen (dilution 1: 2 of the main suspension), thrombin (0.125 units / ml), ristomycin (0.8 mg/ml), adrenaline (5×10^{-6} M), a combination of inductors ADP + adrenaline, ADP + collagen and adrenaline + collagen were used to simulate real blood flow conditions. The morphological intravascular activity of platelets was determined using a phase contrast microscope. The antiaggregation activity of the vessel wall with all inductors used was evaluated against the background of a temporary venous occlusion with the calculation of the index of antiaggregatory activity of the vascular wall (IAAVW). Statistical processing of the results obtained was carried out using Student's t-test. Results are presented as $M \pm m$.

RESULTS

In calves with functional digestive disorders, an increase in the LPO of the plasma was noted. Thus, the concentration of thiobarbituric acid-active products in plasma was 5.10 ± 0.02 $\mu\text{mol/l}$, in the control - 3.92 ± 0.06 $\mu\text{mol/l}$. The level of MDA in platelets was also increased (1.54 ± 0.004 nmol/ 10^9 platelets) and in the control (0.89 ± 0.02 nmol/ 10^9 platelets), which indicate the activation of free-radical oxidation in them due to

the weakening of intraplatelet antioxidant activity. The plasma AHP content in calves with functional digestive disorders was 3.50 ± 0.01 D₂₃₃/1 ml (in the control 1.92 ± 0.02 D₂₃₃/1 ml). In platelets of calves with functional disorders of digestion of AHP (3.49 ± 0.01 D₂₃₃/10⁹ platelets) also significantly exceeded control values (2.87 ± 0.04 D₂₃₃/10⁹ platelets).

Increased LPO in platelets in newborn calves became possible as a result of a significant weakening of the antioxidant enzymes of the blood platelets - superoxide dismutase - 1250.0 ± 4.36 IU/10⁹ platelets (in healthy calves 1780.0 ± 2.06 IU/10⁹ platelets) and catalase 5690.0 ± 21.0 IU/10⁹ platelets (in the comparison group 10500.0 ± 11.05 IU/10⁹ tons of platelets). The level of medium molecules in the plasma at 280 nanomol was 0.49 ± 0.01 conventional units, at 254 nanomol - 0.32 ± 0.02 conventional units, against the control of 0.32 ± 0.002 conventional units and 0.24 ± 0.03 conventional units, respectively. In platelets of calves with functional disorders of the digestive system, average molecules at 280 nanomol - 0.061 ± 0.02 conventional units/10⁹ platelets, with 254 nanomol - 0.069 ± 0.03 conventional units/10⁹ platelets (in the control 0.050 ± 0.04 conventional units/10⁹ platelets and 0.055 ± 0.04 conventional units/10⁹ platelets, respectively).

Evaluation of the lipid composition of platelet membranes in newborn calves with functional digestive disorders revealed a decrease in the total phospholipid content in them to 0.38 ± 0.001 μ mol/10⁹ platelets and an increase in cholesterol level to 0.82 ± 0.001 μ mol/10⁹ platelets. In the control, the analogous indices were 0.49 ± 0.002 μ mol/10⁹ platelets and 0.73 ± 0.001 μ mol/10⁹ platelets, respectively. In calves with functional digestive disorders, an increase in thromboplastin formation was noted. The time of formation of active thromboplastin in them was 2.95 ± 0.01 minutes, the activity - 9.6 ± 0.02 s. In the control group, thromboplastin was formed in 2.40 ± 0.01 minutes, and its activity was 14.0 ± 0.05 s.

In the platelets of newborn calves with functional disorders of the digestive system, the exchange of arachidonic acid in them and the increase in thromboxane formation were noted. Thus, in a simple transfer test, the level of thromboxane in the blood plates of calves was indirectly estimated at $74.3 \pm 0.03\%$ (in the control, $39.2 \pm 0.02\%$). These figures indicate the activation of cyclooxygenase, detected by the reduction of AP in the collagen-aspirin test - $96.8 \pm 0.05\%$ and thromboxane synthetase, determined by the restoration of AP in the collagen-imidazole test - $54.6 \pm 0.02\%$. In healthy animals, similar indicators were 78.4 ± 0.19 and $30.3 \pm 0.01\%$, respectively.

The level of platelets in the blood of calves with functional digestive disorders was within the normal range. At the same time, they had an acceleration of antibodies, especially under the influence of collagen - 25.3 ± 0.20 s. (in the control - 30.0 ± 0.12 s.). Slightly slower AP developed in calves under the influence of ADP (33.0 ± 0.12 s.) and ristomycin (26.2 ± 0.13 s.), in control - 39.0 ± 0.28 s and 41.0 ± 0.26 s, respectively. Thrombin and adrenaline antibodies also developed faster than in controls and were equal in calves to 42.4 ± 0.11 s and 75.6 ± 0.16 s, respectively. The time of AP development under the influence of combined use of inductors was also accelerated. ADP + adrenaline - 20.0 ± 0.12 s., ADP + collagen - 18.0 ± 0.09 s, adrenaline + collagen - 20.3 ± 0.07 s.

When functional disorders of the digestive system in newborn calves on the background of venous occlusion, there was a slowdown of AP, especially pronounced for adrenaline - IAAVW 1.30 ± 0.06 (in the control - 1.65 ± 0.02). A slightly smaller IAAVW is registered for H₂O₂ (1.27 ± 0.07), ristomycin (1.28 ± 0.06) and ADP (1.22 ± 0.05). IAAVW for thrombin and collagen AP were further reduced - 1.18 ± 0.12 and 1.17 ± 0.11 , respectively. The indices of the aggregation activity of the vascular wall with the combination of inductors were also lower than in the control: for ADP + epinephrine 1.25 ± 0.03 s, ADP + collagen - 1.24 ± 0.01 s, adrenaline + collagen - 1.16 ± 0.07 s.

The state of the intravascular activity of platelets in newborn calves with functional digestive disorders was characterized by its increase. Discocytes in the blood of sick calves amounted to $62.0 \pm 0.20\%$ (in the control - $82.0 \pm 0.16\%$). The number of disco-echinocytes increased ($18.0 \pm 0.40\%$). The content of spherocytes, sphero-echinocytes and bipolar forms of platelets also significantly exceeded control values and reached in calves with functional digestive disorders $12.0 \pm 0.03\%$, $6.0 \pm 0.02\%$ and $2.0 \pm 0.01\%$, respectively. The sum of the active forms of platelets with functional digestive disorders in calves was $38.0 \pm 0.30\%$, in the control - $18.0 \pm 0.20\%$, small and large aggregates contained 15.2 ± 0.06 and 4.7 ± 0.03 per 100 free-lying platelets, in control - 3.6 ± 0.04 and 0.12 ± 0.01 per 100 free-lying platelets, respectively, with the number of platelets in the

aggregates in animals with functional digestive disorders reached $14.6 \pm 0.02\%$, against $5.0 \pm 0.20\%$ in the control.

DISCUSSION

Functional digestive disorders in calves are complex and are accompanied by the development of thrombocytopenia and the activation of blood clotting [13,14]. The development of functional disorders of the digestive system causes shifts in the ratio of cholesterol / phospholipids in the membranes of platelets [15,16], which together with digestive disorders [17] and absorption [18] promotes an increase in the blood circulation [19] and then in platelets, the content of medium molecules, contributing to the weakening of the antioxidant protection of blood platelets [20,21] and an increase in the concentration of primary and secondary POL products in them [22,23]. Under these conditions, calves activate platelets and thromboplastin formation [24,25]. An increase in the thrombogenic potential of blood plasma with functional disorders of the digestive system is primarily due to the activation of platelet functions [26,27], and not to an increase in the levels of various coagulation factors, including fibrinogen [28]. Fibrin formation activation, which undoubtedly occurs during functional disorders of the digestive system, occurs primarily on the surface of activated platelets [29,30] and is always secondary in relation to their adhesion and aggregation [31,32].

The combination of metabolic shifts, changes in the composition of platelet membranes, an increase in the content of medium molecules and an increase in intraplatelet LPO, which occurs during functional disorders of the digestive system, leads to an increase in intravascular platelet activity [33,34], increasing the content of active platelets in the bloodstream [35]. High intravascular activity of platelets leads to increased platelet aggregation activity under the influence of various inducers [36,37]. Possible mechanisms of this enhancement are activation of arachidonic acid metabolism with an increase in thromboxane formation [38], registered in transfer samples, and an increase in the concentration of von Willebrand factor [39] indirectly estimated by acceleration of AP with ristomycin [40].

All newborn calves with functional disorders of the digestive system showed a significant decrease in IAAVW compared to healthy calves, which is explained by a decrease in the production of antiplatelet agents in the vessel walls and, above all, prostacyclin [41].

The formation of functional digestive disorders is accompanied not only by the development of thrombocytopenia, but also by a weakening of the functions of the vascular wall [42]. Changes in the lipid composition of platelet membranes entail activation of platelets, which, together with other components of functional disorders of the digestive system, contribute to the weakening of the antiaggregatory activity of the vascular wall, leading to an increase in the intravascular AP [43]. High platelet aggregation activity [44] under the influence of various inductors indicates an increased activity of platelets in vivo [45]. In this case, arachidonic acid metabolism is weakened in the vessel wall, where its main metabolite is a vasodilator and antiplatelet agent - prostacyclin - the main thromboxane antagonist [46,47].

A simultaneous study of the effect of several inductors on the AP process without venous occlusion and against its background in calves with functional digestive disorders showed the reciprocal effect of agonists on platelets with low sensitivity of the latter to the disaggregating signals of the vascular wall in actual blood flow conditions [48-50]. AT registration against the background of temporary ischemia and without it under the influence of a combination of inductors allows one to come closer to understanding the actual conditions of blood flow in newborn calves with functional disorders of the digestive system and indicates a high risk of microthrombus formation in them [51-53].

CONCLUSION

In newborn calves with functional digestive disorders, an increase in platelet aggregation functions is observed in vitro and in vivo due to developing changes in the lipid composition of platelet membranes, an increase in the level of middle molecules in them, activation of plasma lipid peroxidation and platelets, increased von Willebrand factor synthesis the weakening of prostacyclin formation and the intensification of thromboxane formation in the blood plates.

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