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The Functional State Of Primary Hemostasis In Newborns Calves With Dyspepsia.

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ABSTRACT

The study of disorders of primary hemostasis in newborn calves with dyspepsia is of practical importance, as its condition plays a leading role in enhancing hemostasis in general, increasing viscosity and worsening blood rheology with a tendency to intravascular thrombosis. At the same time, impaired platelet aggregation capacity, vascular wall antiaggregation activity and blood platelet intravascular activity in newborn calves with dyspepsia are poorly understood. In newborn calves with dyspepsia, an increase in platelet aggregation function was found in vitro and in vivo. These disorders are based on deep changes in the lipid composition of platelet membranes, an increase in the level of medium molecules in plasma and blood plates, activation of lipid peroxidation in them, increased synthesis in the vascular wall of von Willebrand factor and intensification of thromboxane formation in blood plates. The resulting activation of thromboplastin formation is the leading cause of increased blood coagulation in newborn calves with dyspepsia. In this regard, the correction of disorders of platelet hemostasis in these calves should include a pathogenetically determined complex that can reduce the level of middle molecules in the body and eliminate dyspepsia. **Keywords**: platelets, newborn calves, dyspepsia, physiology, hemostasis.

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INTRODUCTION

The work of primary hemostasis is extremely important for the whole organism at all stages of ontogenesis [1,2,3]. The study of disorders of primary hemostasis in newborn calves with dyspepsia is of practical importance [4,5], since it is the activation of primary hemostasis that plays a leading role in the activation of hemostasis in general [6,7], increase of viscosity and deterioration of blood rheology [8,9] with a tendency to intravascular thrombosis [10-15]. At the same time, violations of the aggregation ability of platelets, the antiaggregation activity of the vascular wall, and the intravascular activity of platelets in newborn calves with dyspepsia have been very poorly studied [16,17]. The degree of disturbance in platelet dysfunction in newborn calves with dyspepsia of the lipid composition of their membranes, the level of peroxidation and antioxidant protection of platelets, as well as the level of arachidonic acid exchange in them is not determined. There is fragmentary information that dyspepsia is accompanied in newborn calves by an increase in the plasma level of medium molecules capable of disrupting many functions of the body. The extent of the increase in average molecules in platelets, contributing in many ways to the formation of thrombocytopathy, has not been elucidated.

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

MATERIALS AND METHODS

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 March 18, 1986, and confirmed in Strasbourg June 15, 2006) and approved by the local ethic committee of Russian State Social University (Record Nº12 dated December 3, 2015).

Under the supervision there were 153 newborn calves with dyspepsia 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 middle molecules in plasma and washed and resuspended platelets was determined. Plasma lipid peroxidation (LPO) activity was determined by the content of thiobarbituric acid-active products by the Agat-Med company kit, acyl hydroperoxides (AHP), and the intraplatelet lipid peroxidation by the concentration of the basal level of malondialdehyde (MDA) in the reduction of heartbroken pattern. The intra-platelet antioxidant system characterized the activity of catalase and superoxide dismutase.

The cholesterol level 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) using a photoelectrocolorimeter were used. The number of platelets in capillary blood in the Goryaev chamber was counted. The aggregation ability of platelets was studied 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) to simulate real blood flow conditions, combinations of inductors ADP + adrenaline, ADP + collagen and adrenaline + collagen are used. 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 dyspepsia, an increase in plasma LPO was noted. Thus, the concentration of thiobarbituric acid-active products in plasma was $5.10\pm0.02~\mu$ mol/l, in the control - $3.92\pm0.06~\mu$ mol/l. The level of MDA in platelets was also increased ($1.54\pm0.004~\mu$ mol/ $10^9~\mu$ platelets) And in the control ($0.89\pm0.02~\mu$ mol/ $10^9~\mu$ platelets), which indicate the activation of free-radical oxidation in them due to weakening



intraplatelet antioxidant activity. The content of AHP in the plasma of sick calves was $3.50\pm0.01\ D_{233}/1\ ml$ (in the control $1.92\pm0.02\ D_{233}/1\ ml$. In the platelets of patients with AHP ($3.49\pm0.01\ D_{233}/10^9\ platelets$) also significantly exceeded the control values ($2.87\pm0.04\ D_{233}/10^9\ platelets$).

Activation of free-radical oxidation in platelets in sick calves became possible as a result of a significant weakening of the antioxidant enzymes of the blood platelets - superoxide dismutase - $1250.0\pm4.36\ IU/10^9$ platelets (in healthy calves, $1780.0\pm2.06\ IU/10^9$ platelets) and catalase - $5690.0\pm21.0\ IU/10^9$ platelets (in the comparison group $10500.0\pm11.05\ IU/10^9$ platelets). The level of medium molecules in plasma at 280 nanomol amounted to 0.49 ± 0.01 conventional units, with 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, calves with medium molecules dyspepsia were at 280 nanomol - 0.061 ± 0.02 conventional units/ 10^9 platelets, with 254 nanomol - 0.069 ± 0.03 conventional units/ 10^9 platelets (in the control 0.050 ± 0.04 conventional units/ 10^9 platelets and 0.055 ± 0.04 conventional units/ 10^9 platelets, respectively).

Determination of the lipid composition of platelet membranes in calf patients revealed a decrease in total phospholipid content in them to $0.38\pm0.001~\mu\text{mol}/10^9$ platelets and an increase in cholesterol level to $0.82\pm0.001~\mu\text{mol}/10^9$ platelets. In the control, the analogous indices were $0.49\pm0.002~\mu\text{mol}/109$ platelets and $0.73\pm0.001~\mu\text{mol}/10^9$ platelets, respectively. In sick animals, an increase in thromboplastin formation was noted. The time of formation of active thromboplastin in them was $2.95\pm0.01~\text{min}$, activity $-9.6\pm0.02~\text{sec}$. In the control group, thromboplastin was formed in $2.40\pm0.01~\text{min}$, and its activity was $14.0\pm0.05~\text{s}$.

The combination of biochemical changes in platelets characterized the increased exchange of arachidonic acid in them and the increase in thromboxane formation. In a simple transfer test, the level of thromboxane in the blood plates of calves was indirectly estimated at $74.3\pm0.03\%$ (in the control, $39.2\pm0.02\%$). These figures indicate the activation of cyclooxygenase, detected by the reduction of AP in the collagen-aspirin test - $96.8\pm0.05\%$ and thromboxane synthetase, determined by the restoration of AP in the collagen-imidazole test - $54.6\pm0.02\%$. In healthy animals, similar indicators were 78.4 ± 0.19 and $30.3\pm0.01\%$, respectively.

The concentration of platelets in the blood of patients was within the normal range. AP acceleration was noted, 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 (p <0.01). 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.

In patients with calves on the background of venous occlusion, there was a slowdown in AP, especially pronounced for adrenaline - IAAVW 1.30 ± 0.06 s (in the control - 1.65 ± 0.02 s). A slightly smaller IAAVW is registered for H_2O_2 (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, epinephrine + collagen - 1.16 ± 0.07 c.

The intravascular activity of platelets of sick animals was characterized by its increase. Discocytes in the blood of sick calves amounted to $62.0\pm0.20\%$ (in the control - $82.0\pm0.16\%$). The number of discoechinocytes increased ($18.0\pm0.40\%$). The contents of spherocytes, sphero-echinocytes and bipolar forms of platelets also significantly exceeded the control values and reached $12.0\pm0.03\%$, $6.0\pm0.02\%$ and $2.0\pm0.01\%$ in sick calves, respectively. The sum of the active forms of patients' platelets was $38.0\pm0.30\%$, in the control - $18.0\pm0.20\%$, small and large aggregates contained 15.2 ± 0.06 and 4.7 ± 0.03 , control - 3.6 ± 0.04 and 0.12 ± 0.01 , respectively, with the number of platelets in the aggregates in sick animals reached $14.6\pm0.02\%$, against $5.0\pm0.20\%$ in the control.

DISCUSSION

Dyspepsia in calves is complex and is accompanied by the development of thrombocytopathy and the activation of the blood coagulation process [18,19]. The pathogenesis of dyspepsia causes shifts in the cholesterol / phospholipid ratio in platelet membranes [20–25], which, together with digestive disorders [26]

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and absorption, increases in the bloodstream [27–30] and then in platelets CM content [31] causing a weakening of the antioxidant protection of blood plates and an increase in the concentration of primary and secondary POL products in them [32,33]. Under these conditions, calves activate platelets and thromboplastin formation [34-37]. An increase in the thrombogenic potential of blood plasma during dyspepsia is associated primarily with the activation of platelet functions [38-40], and not with an increase in the levels of various coagulation factors, including fibrinogen [41]. Fibrin formation, undoubtedly occurring during dyspepsia, occurs primarily on the surface of activated platelets and is always secondary in relation to their adhesion and aggregation [42,43].

A combination of metabolic disorders, changes in the composition of platelet membranes, an increase in the content of medium molecules in them and an increase in intraplatelet lipid peroxidation resulting in dyspepsia increases the intravascular activity of platelets, increasing the content of active platelets in the bloodstream [44]. The high intravascular activity of platelets causes an increase in platelet aggregation under the influence of various inducers [45]. Possible mechanisms of this enhancement include activation of arachidonic acid exchange with an increase in thromboxane formation in them, registered in transfer samples, and an increase in the concentration of von Willebrand factor participating in the aggregation process, indirectly assessed by acceleration of AP with ristomycin [46,47].

In all cases, sick animals showed a significant decrease in IAAVW compared with healthy people, which is explained by a reduction in the production of antiplatelet agents in the vascular walls, primarily prostacyclin [48].

Disturbances in dyspepsia are complex and are accompanied not only by the development of thrombocytopathy, but also by a weakening of the functions of the vascular wall [49]. Changes in the lipid composition of platelet membranes entail activation of platelets, which, together with other components of dyspepsia, contribute to the weakening of the antiaggregatory activity of the vascular wall, leading to an increase in intravascular AP [50,51]. High platelet aggregation activity under the influence of various inductors indicates an increased activity of platelets in vivo. 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 [52,53].

The study of the combined effect of inductors on the AP process without venous occlusion and on its background in sick calves showed a potentiopatinating effect of agonists on platelets with a low sensitivity of the latter to disaggregating signals of the vascular wall under actual blood flow conditions. The registration of AP 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 dyspepsia and indicates a high risk of thrombosis in them.

The revealed violations of platelet hemostasis in calves with dyspepsia require adequate correction aimed at breaking the "vicious circles" developing in dyspepsia.

CONCLUSION

In newborn calves with dyspepsia, an increase in platelet aggregation function was found in vitro and in vivo. These disorders are based on changes in the lipid spectrum of platelet membranes, an increase in the level of medium molecules in them, activation of plasma lipid peroxidation and platelets, increased von Willebrand factor synthesis in the vascular wall with weakening of prostacyclin formation and intensification of thromboxane formation in blood plates. Activation of thromboplastin formation is the leading cause of increased blood coagulation in newborn calves with dyspepsia. Correction of disorders of platelet hemostasis should include a pathogenetically determined complex that can treat dyspepsia and optimize blood rheology simultaneously.

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