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Dynamics Of The Functional State Of Platelet Functions In Newborn Calves Receiving Correction For Dyspepsia.

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ABSTRACT

Shifts in platelet hemostasis in newborn calves with dyspepsia contribute to the development of intravascular thrombosis in them. At the same time, approaches to the rapid effective correction of the functional state of platelets in calves with dyspepsia with the complete elimination of the risk of thrombosis have not yet been developed. Currently, in practice, use the drug - phosphopag, which is able to effectively arrest dyspepsia in newborn calves. An assumption was made about the possible efficacy of this substance in terms of the correction of platelet dysfunction in newborn calves with dyspepsia when combined with the ecos sorbent and calcium gluconate. In newborn calves with dyspepsia, an increase in platelet aggregation function was found in vitro and in vivo. These disorders are based on violations of cholesterol and phospholipid ratios in platelet membranes, an increase in the level of medium molecules in plasma and blood plates, activation of lipid peroxidation in them, increased synthesis of von Willebrand factor in the wall of blood vessels and an increase in thromboxane formation in blood plates. Platelet activation is the leading cause of increased blood coagulation in newborn calves with dyspepsia. The use of a complex of phosphopag, ekos and calcium gluconate in calves with dyspepsia reduced the activity of lipid peroxidation and the content of middle molecules in their blood and platelets. The use of phosphopag, ekos and calcium gluconate in newborn calves with dyspepsia for 10 days normalizes the state of platelet aggregation ability in vitro and their intravascular activity. Correction of violations of platelet hemostasis with the help of a complex of phosphopagus, ekos and calcium gluconate by reducing the level of middle molecules in the body and suppressing lipid peroxidation.
Keywords: calves, dyspepsia, thrombocytopathy, phosphopag, ekos, calcium gluconate.

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INTRODUCTION

Shifts in platelet hemostasis in newborn calves with dyspepsia contribute to the development of intravascular thrombosis in them [1,2,3]. Activation of primary hemostasis in dyspepsia depends largely on the enhancement of lipid peroxidation (POL) in animals [4,5], leading to the launch of intra-platelet mechanisms to enhance their aggregation [6,7]. Approaches to rapid effective correction of the functional state of platelets in calves with dyspepsia with the complete elimination of the risk of thrombosis have not yet been developed [8-10].

Currently, in practice, the drug is used - phosphopag (polyhexamethylene guanidine phosphate), which is able to effectively arrest dyspepsia in newborn calves. This becomes possible with the joint use of drugs of different actions. It was suggested that a possible increase in the effectiveness of this substance for the correction of platelet dysfunction in newborn calves with dyspepsia using a combination of phosphopag with ekos (hydroaluminosilicate) sorbent and calcium gluconate.

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 №12 dated December 3, 2015).

The study group consists of 26 newborn calves with dyspepsia. Patients calves showed all signs of dyspepsia with bright intoxication. The control group consisted of 267 healthy newborn calves. The survey included the determination of the activity of plasma lipid peroxidation (LPO) by the content of thiobarbituric acid-active products using the Agat-Med kit. The antioxidant potential of the liquid part of the blood, reflecting the level of antioxidant blood vitamins in the blood, was evaluated. Intra-platelet lipid peroxidation was found by the concentration of the basal level (without prooxidative stimulation) of malonic dialdehyde (MDA) in the reduction reaction with thiobarbituric acid. The level of medium molecules in plasma and washed, resuspended platelets was determined. The number of platelets in capillary blood in the Goryaev chamber was counted. Platelet aggregation (AP) was studied by a visual micromethod using as inducers ADP (0.5×10^{-4} M), collagen (in a standard concentration, a 1:2 dilution of the main suspension), thrombin (0.125 units / ml), ristomycin (0.8 mg/ml), adrenaline hydrochloride (5×10^{-6} M, pH -7,4), as well as combinations of ADP and adrenaline, ADP and collagen, adrenaline and collagen to simulate real blood flow conditions. All inductors solutions were prepared each time before use. The intravascular activity of platelets was determined visually using a phase contrast microscope.

All 26 calves were given 0.01% phosphopag (polyhexamethylene guanidine phosphate) 100.0 ml each in the morning, 10% calcium gluconate 10.0 ml each for lunch and ecos (hydroaluminosilicate) 150 mg/kg body weight in the evening for 10 days, including in the scheme of feeding. Statistical processing of the results obtained was carried out using Student's t-test.

RESULTS

In newborn calves with dyspepsia, there was an increase in lipid peroxidation, while the concentration of thiobarbituric acid-active products in plasma was 5.16 ± 0.12 µmol/l, in the control - 3.92±0.06 µmol/l. The antioxidant activity of the plasma of sick animals was reduced (21.2±0.06%), in the control - 28.6±0.04%. The level of MDA in platelets was increased (1.66±0.001 nmol/10^9 platelets), in the control - 0.89±0.02 nmol/10^9 platelets), which indicated the activation of free-radical oxidation in them due to the weakening of intraplatelet antioxidant activity. The levels of medium molecules in plasma were Average molecules 280 - 0.52±0.04 conventional units, Average molecules 254 - 0.35±0.02 conventional units, the content of middle molecules in platelets. Average molecules 280 - 0.065±0.02 conventional units/10^9 platelets, Medium molecules 54 - 0.072±0.01 conventional units/10^9 platelets, significantly exceeding control values.

The use of a combination of phosphopag, ekos and calcium gluconate in calves had a positive effect on the LPO of plasma and platelets. The content of thiobarbituric acid-active plasma products decreased ($p < 0.01$). On the 10th day of treatment, their concentration was 3.97±0.05 µmol/l. With a decrease in POL

products, normalization of the average molecules 280 to 0.32 ± 0.09 conventional units is noted, the average molecules 254 - 0.23±0.06 conventional units. The decrease in the severity of plasma LPO was parallel with the decrease in the basal level of MDA in platelets after the 10th day of treatment (0.90±0.03 nmol/10^9 platelets). With the combined appointment of calves of phosphopagus, ekos and calcium gluconate, the average molecules 280 - 0.050±0.06 conventional units /10^9 platelets normalized in platelets, the average molecules 254 - 0.054±0.01 conventional units/10^9 platelets.

The content of platelets in the blood of sick calves before and after treatment was within the normal range. In calves with dyspepsia, acceleration of antibodies was found, especially under the influence of collagen (20.3±0.05 s), before prescribing therapy. Slightly slower AP developed in sick calves under the influence of ADP (36.0±0.10 s) and ristomycin (31.6±0.02 s). Thrombin (43.6±0.22 s) and adrenaline (82.0±0.03 s) antibodies occurred later, but developed faster than controls (54.0±0.02 s and 97.0±0.45 s, respectively) ($p<0.01$). The time of AP development under the influence of the combined use of inductors was also accelerated (ADP + adrenaline - 22.0±0.05 s, ADP + collagen - 20.0±0.01 s, adrenaline + collagen - 19.0±0.02 s).

When prescribing a combination of phosphopag, ekos and calcium gluconate, the AP time increased under the influence of all inductors. On the 10th day of treatment with the most active inducer of AT, they retained collagen (31.0±0.05 s). Were less active - ADP (39.0±0.05 s), ristomycin (41.0±0.12 s). Later, AP developed under the influence of thrombin and adrenaline. AP time was prolonged when inductors were combined (ADP + adrenaline - 36.0 ± 0.03 s, ADP + collagen - 27.0±0.05 s, adrenaline + collagen - 30.0±0.05 s).

In calves with dyspepsia, an increase in intravascular platelet activity was observed. The level of discocytes, determined visually with phase contrast, in the blood of sick calves was 62.3±0.06% (in the control - 82.0±0.16%). The content of disco-echinocytes was increased 1.6 times. The number of spherocytes and sphero-echinocytes also significantly exceeded the control values (13.2±0.04% and 6.8±0.05%, respectively). The amount of active forms (the sum of platelet cells, spheroechinocytes, spherocytes and bipolar forms) in the bloodstream (37.7±0.02%) of sick calves was increased 2.09 times, against control (18.0±0.02%). Small and large aggregates in their blood contained 16.2±0.05 per 100 free-lying platelets and 45.5±0.02 per 100 free-lying platelets, 4.5 and 45.8 times the control values (3.6±0.04 and 0.12±0.01 per 100 free-lying platelets, respectively), and the number of platelets in the aggregates in sick animals (14.0±0.02%) exceeded the control by 2.8 times (5.0± 0.2%).

The purpose of the combination of phosphopag, ekos and calcium gluconate to calves with dyspepsia sick allowed to reduce the intravascular activity of platelets. By the end of the 10-day treatment, a significant increase in inactive forms of platelets, as well as discoid platelets in the blood of patients with the use of phosphopag, ekos and calcium gluconate increased to 82.3 ± 0.6%. At the end of treatment, the levels of disco-echinocytes, spherocytes and sphero-echinocytes in the blood of animals significantly decreased under the influence of treatment (9.4±0.01%, 4.6±0.05% and 2.7±0.3%, respectively). The sum of the active forms of platelets during therapy with phosphopag, ekosom and calcium gluconate (17.7±0.04%) corresponded to the control (18.0±0.2%). The number of small and large aggregates by 10 days of treatment significantly decreased by 4.37 and 36.6 times, respectively. The number of platelets in the aggregates was significantly reduced to 5.0±0.09%, equaling the control level (5.0±0.2%).

DISCUSSION

Enhanced POL in platelets of calves and dyspepsia indicates a decrease in the antioxidant activity of their liquid blood [11–15] and causes an inevitable and explainable increase in the plasma and platelets level of middle molecules [15–20]. Normalization of peroxidation and an increase in the antioxidant potential of plasma with a decrease in the average molecules during treatment indicate a significant normalizing effect of the combined use of phosphopag, ecos and calcium gluconate on homeostasis in newborn calves with dyspepsia [21,22]. Their effects are mediated by their antimicrobial properties (phosphopag), sorption of toxins (ecos), and an increase in the antioxidant potential of the liquid part of the blood, increasing the functionality of body cells [23,24], including the gastrointestinal tract of the calf (phosphopag and calcium gluconate) [25-30] according to the research.

Normalization of the estimated hemostasis in calves against the background of the combined use of phosphopagus, ekos and calcium gluconate indicates its positive effect on the mechanisms of platelet hemostasis in newborn calves with dyspepsia [31,32]. Undoubtedly, this is due to the correction of clinical manifestations of dyspepsia, decreased levels of POL and average molecules in plasma and platelets with a decrease in platelet sensitivity to aggregation inductors [33-38], which can be judged by the increase in their aggregation time [39-41]. The activity of platelet aggregation in newborn calves with dyspepsia when administered for a 10-day period of phosphopag, ekos and calcium gluconate corresponded to the control group [42-45].

Achieving the normal duration of AT under the influence of ristomycin in newborn calves who were on therapy with phosphopagus, ecosomes and calcium gluconate indicates the optimization of the concentration in the blood of the adhesive molecule - von Willebrand factor [46,47].

Full normalization of intravascular platelet activity in the treatment of phosphopagus, ecosomes and calcium gluconate in newborn calves with dyspepsia helps to normalize microcirculation, since the content of active platelet forms and their aggregates is no longer able to block the capillaries at the level achieved [48,49]. Given the effectiveness of the correction of platelet disorders in newborn calves with dyspepsia, the treatment can be recommended [50,51], the studied combination of drugs for widespread use in livestock farms [52,53].

CONCLUSION

The use of a complex of phosphopag, ekos and calcium gluconate in calves with dyspepsia reduces the activity of lipid peroxidation and the content of medium molecules in their blood and platelets. The use of phosphopag, ekos and calcium gluconate in neonatal calves with dyspepsia for 10 days normalizes the state of the estimated indices of platelet functions, optimizing the aggregation ability of platelets and the intravascular activity of the platelets.

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