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The Dynamics Of The Physiological Properties Of Hemostasis In Newborn Calves With Functional Disorders Of The Digestion Against The Background Of Their Consumption Of Needles Extract.

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ABSTRACT

Dyspeptic disorders in newborn calves are still a common condition. At the same time, it is quite often that there are violations in the hemostasis system, contributing to the onset of intravascular thrombosis in them. In a study in calves with dyspepsia that occurred during the neonatal period, an increase in platelet aggregation ability *in vitro* and *in vivo* was found. They are based on pronounced changes in the fatty composition of the membranes of blood platelets, the increase in the number of medium molecules in plasma and platelets, the enhancement of lipid peroxidation in them, the intensification of the formation of von Willebrand factor vessels. Increased thromboxane formation can be considered a serious reason for the increased activity of hemostasis in calves in the neonatal phase with dyspepsia. Previously conducted research on a highly biologically active drug, fosprenil, which is based on needle polyprenols, has shown its ability to influence metabolism, simulate natural resistance, as well as anti-inflammatory activity, properties of the hepatoprotector and detoxicant. The presence of these effects prompted the author to use fosprenil in this study. Appointment of fosprenil in calves with functional digestive disorders was able to improve lipid peroxidation and reduce the content of medium molecules in their blood and platelets. A ten-day application of fosprenil in newborn calves with functional disorders of the digestive system also improved the state of the estimated indicators of primary hemostasis, optimizing the aggregation ability of platelets and the intravascular activity of the blood platelets.

Keywords: newborn calves, platelets, fosprenil, functional disorders of the digestive system.

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INTRODUCTION

Dyspeptic disorders in newborn calves are still a common condition [1,2]. At the same time, violations of the hemostasis system [3,4], which contribute to the onset of intravascular thrombus formation [5–8], are very often observed. Strengthening the functioning of primary hemostasis, including in functional digestive disorders, is associated with an increase in lipid peroxidation (LPO) in their bodies [9-12], leading to the launch of intracellular mechanisms for the realization of their aggregation function [13-16]. To this day, approaches to the effective elimination of increased functional activity of platelets in calves with functional digestive disorders with the complete elimination of the risk of thrombosis have not been clarified.

The study of a new highly biologically active drug - fosprenil, which is based on needle polyprenols, revealed its ability to influence metabolism, simulate natural resistance, having anti-inflammatory activity, being a hepatoprotector and detoxifier. An assumption was made about the possibility of eliminating platelet activation in calves in the neonatal phase with functional disorders of digestion using fosprenil.

The aim of the work is to determine the possibility of effective correction of platelet hemostasis disorders in newborn calves with functional disorders of the digestive system through the use of fosprenil.

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).

26 newborn calves with functional digestive disorders were taken into work. In calves with functional digestive disorders, dyspepsia was observed with bright intoxication. The control group is represented by 267 healthy newborn calves.

The activity of LPO in plasma was determined during the determination of the level of thiobarbituric acid-active products using the Agat-Med kit. Figured out the antioxidant activity of plasma. Intra-thrombocytic POL was determined by the level of malondialdehyde (MDA) in the course of reduction with thiobarbituric acid. Concentrations of medium molecules in the blood and washed, resuspended blood plates were recorded. The number of platelets in the blood in the Goryaev chamber was counted. The state of platelet aggregation (AP) was determined visually in response to inductors of ADP (0.5×10^{-4} M), thrombin (0.125 U/ml), collagen (dilution 1:2 of the main suspension), ristomycin (0.8 mg/ml), adrenaline (5×10^{-6} M) and with combinations of ADP and adrenaline, adrenaline and collagen, ADP and collagen. The state of intravascular platelet activity was assessed visually using a phase contrast microscope.

All 29 calves were given 0.8 ml/kg fosprenil intramuscularly for 10 days. Mathematical processing of the results carried out by t-student criterion.

RESEARCH RESULTS

In animals with functional digestive disorders, a high level of lipid peroxidation was found: the amount of thiobarbituric acid products in the blood was 5.16 ± 0.12 $\mu\text{mol/l}$ (in the control - 3.92 ± 0.06 $\mu\text{mol/l}$). Antioxidant protection of plasma calves with functional digestive disorders was weakened ($21.2 \pm 0.06\%$), in the control ($28.6 \pm 0.04\%$). The number of MDA in the blood 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 LPO in connection with the depression of intrathromycin antioxidant protection. The concentration of medium molecules in the blood was equal to Average molecules 54 - 0.35 ± 0.02 conventional units and Average molecules 280 - 0.52 ± 0.04 conventional units, the number of middle molecules in the blood plates Average molecules 254 - 0.072 ± 0.01 conventional units/ 10^9 platelets and Average molecules 280 - 0.065 ± 0.02 conventional units/ 10^9 platelets significantly prevailed over control values.

The administration of fosprenil to calves had a positive effect on the LPO in the liquid part of the blood and platelets. The number of thiobarbituric acid products in the blood decreased ($p < 0.01$). After 10 days of

correction, their level was $4.43 \pm 0.06 \mu\text{mol/l}$. With a decrease in lipid peroxidation products in the blood, normalization of the average molecules of 254 - 0.29 ± 0.05 conventional units and average molecules of 280 to 0.41 ± 0.11 conventional units was found. The decrease in the level of POL in plasma was in parallel with the decrease in the level of MDA in the blood plates after 10 days of correction ($1.20 \pm 0.06 \text{ nmol}/10^9$ platelets). During the administration of fosprenil to calves, the number of Average molecules 254 – 0.063 ± 0.04 conditional units/ 10^9 platelets and Average molecules 280 – 0.056 ± 0.07 units/ 10^9 platelets decreased in the blood platelets.

In calves with functional disorders of the digestive system, prior to the administration of exposure, an acceleration of antibodies was detected, most pronounced in response to collagen (20.3 ± 0.05 s). Later, AP occurred in calves with functional digestive disorders with ADP (36.0 ± 0.10 s) and ristomycin (31.6 ± 0.02 s). AP with thrombin (43.6 ± 0.22 s) and adrenaline (82.0 ± 0.03 s) occurred later, but before the control ($p < 0.01$). The duration of occurrence in response to combinations of inductors was also accelerated (ADP + collagen - 20.0 ± 0.01 s, ADP + adrenaline - 22.0 ± 0.05 s, adrenaline + collagen – 19.0 ± 0.02 s) .

During the correction with fosprenil, the AP time increased in response to inductors. After 10 days of treatment, collagen was the strongest inducer of AP (28.0 ± 0.03 s). Ristomycin (36.2 ± 0.14 s) and ADP (37.0 ± 0.02 s) were weaker. Later, AP appeared under the influence of thrombin and adrenaline. The AP time increased in response to inductors combinations (ADP + collagen - 24.0 ± 0.07 s, ADP + adrenaline - 26.0 ± 0.06 s, adrenaline + collagen - 25.0 ± 0.04 s).

The intravascular activity of platelets in calves with functional disorders of the digestive system was characterized by its increase. The level of discocytes in calves with functional digestive disorders was $62.3 \pm 0.06\%$ (in the control - $82.0 \pm 0.16\%$). The number of disco-echinocytes was increased by 1.60 times. The number of sphero-echinocytes and spherocytes also significantly exceeded the control ($13.2 \pm 0.04\%$ and $6.8 \pm 0.05\%$, respectively). The total number of active platelets ($37.7 \pm 0.02\%$) in calves with functional digestive disorders was 2.09 times higher than control. Small and large aggregates in their blood were 4.5 and 45.8 times more than in the control, respectively, whereas the number of platelets in the calves with functional digestive disorders was 2.8 times higher than the control.

The use of a combination of fosprenil in patients with functional disorders of the digestion of calves allowed to achieve a positive dynamics of intravascular platelet activity. By the end of the 10 day treatment, a significant improvement in the indicators of intravascular platelet activity was revealed. The number of discoid platelets in calves with functional digestive disorders on the background of correction increased to $72.6 \pm 0.21\%$. The values of disco-echinocytes, sphero-echinocytes and spherocytes in the blood of calves with functional disorders of the digestive system decreased significantly under the influence of treatment ($9.4 \pm 0.01\%$, $4.6 \pm 0.05\%$ and $3.2 \pm 0.16\%$, respectively). The total number of active forms of blood platelets on the background of fosprenyl ($27.4 \pm 0.09\%$) decreased. The number of small and large aggregates after 10 days of correction significantly decreased by 3 and 25 times, respectively. The number of platelets in the aggregates reached $8.6 \pm 0.12\%$.

DISCUSSION

The activation of lipid peroxidation processes in the blood and platelets of animals during functional disorders of the digestive system indicates a decrease in the activity of the antioxidant system of their body [17–20] and causes an increase in the level of middle molecules in the blood and blood plates [21]. Normalization of peroxidation and the growth of plasma antioxidant activity with a decrease in average molecules as a result of exposure indicates a significant normalizing effect [22,23] of combined use of fosprenil on homeostasis in newborn calves with functional disorders of digestion. Their effects are mediated by the impact of all funds on metabolism and increased activity of antioxidant enzymes of the body [24-29].

Normalization of the hemostasis parameters taken into account in animals during the application of fosprenil indicates its positive effect on the mechanisms of ensuring platelet hemostasis [30-32] in calves during the neonatal phase with functional digestive disorders. Obviously, this is due to the improvement of metabolic processes [33-37], reduction of the toxic effect of LPO [38] and medium molecules [39,40] in plasma and blood cells, including platelets, with optimization of the process of exogenous signals reception [41,42].

The state of platelet aggregation in the observed calves with functional disorders of the digestive system, if used for 10 days with fosprenil, approached the control group.

Optimization of the time of onset of AP in response to ristomycin in newborn calves treated with fosprenil indicates a decrease in the blood concentration of the adhesive molecule, von Willebrand factor [43-46].

The decrease in the intravascular activity of platelets in the treatment of fosprenil in newborn calves with functional disorders of the digestion allows to reduce to a minimum the stimulation of hemostasis in general [47-50]. Given the high efficacy of the correction of hemostasis in the observed animals with functional digestive disorders receiving the applied correction, the tested agent can be recommended for widespread use in animal husbandry [51-53].

CONCLUSION

Appointment of fosprenil in calves with functional digestive disorders improves lipid peroxidation and the content of medium molecules in their blood and platelets. A ten-day application of fosprenil in newborn calves with functional digestive disorders improves the state of the estimated indicators of primary hemostasis, optimizing the aggregation ability of platelets and the intravascular activity of the blood platelets.

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