

PROMPT EFFECTIVE CORRECTION OF THROMBOCYTE HEMOSTASIS DIATURBANCES IN NEW-BORN CALVES WITH DYSPEPSIA

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1. Phosphopag, acos and calcium gluconate complex application in new-born calves with dyspepsia normalizes lipid peroxidation and MM content in their blood and thrombocytes.

2. Phosphopag, acos and calcium gluconate application during 10 days in new-born calves with dyspepsia normalizes the condition of the estimated indicators of primary hemostasis, optimizing thrombocyte aggregation and blood platelets' intravascular activity.

Thrombocyte hemostasis alterations in new-born calves with dyspepsia cause the development of intravascular thrombosis. Primary hemostasis activation on dyspepsia mostly depends on lipid peroxidation (LP) increase in animal' organism that activates thrombocyte aggregation increase mechanisms. The ways of prompt and effective correction of thrombocyte functional condition in calves with dyspepsia completely excluding the risk of thrombosis still have not been worked out.

Phosphopag (Polyhexamethylenguanidine phosphate), new biologically active preparation can effectively arrest dyspepsia in new-born calves and is being actively tested now [4]. Much attention is paid to economically available sorbent acos (hydroaluminum silicate from Belgorod region fields). Calcium gluconate is used to stimulate the calves' gastric secretion. The combination of phosphopag, acos and calcium gluconate has been supposed to completely correct thrombocyte function in new-born calves with dyspepsia.

The aim of the research is to find the possibility of prompt effective correction of thrombocyte hemostasis in new-born calves with dyspepsia with the help of the

combination of phosphopag, acos and calcium gluconate.

Materials and methods

Research group is represented by 26 new-born calves with dyspepsia. The sick calves had all the symptoms of dyspepsia with clear intoxication. The control group consisted of 267 healthy new-born calves. The examination comprised the reveal of lipid peroxidation (LP) activity of plasma by the content of TBA-active products with the help of the tool kit of LLC "Agat-Med". Antioxidant potential of liquid part of blood was estimated [1]. Thrombocyte lipid peroxidation was defined by the concentration of basal level of malonic dialdehyde (MDA) in reducing reaction of thiobarbituric acid [7], in modification [3]. The level of medium molecules (MM) was defined in plasma and in resuspended thrombocytes [2]. The thrombocyte amount in capillary blood was calculated in Gorjaev's chamber. The thrombocyte aggregation (TA) was examined by means of visual micromethod [6] using ADP ($0,5 \times 10^{-4}$ M), collagen (the basic suspension dilution 1:2), thrombin (0,125 unit/ml), Ristomycin (0,8 mg/ml) (NPO "Renam"), adrenalin (5×10^{-6} M., Gedeon Richter factory) as inductors and using the combination of ADP and adrenalin, ADP and collagen, adrenalin

and collagen to model the real blood flow condition. Intravascular thrombocyte activity (ITA) was defined with the help of phase-contrast microscope [5] according to Shitikova A.S. and co-authors (1997). According to the bottle-feeding schedule the 26 calves were prescribed to take 100,0 ml of 0,01 % phosphopag in the morning, 10 % 10,0 of calcium gluconate in the afternoon and 150 mg of acos per kg of live weight during 10 days. Statistical treatment of the results achieved was carried out using Student t-criterion.

The results of research

The increase of lipid peroxidation was observed in new-born calves with dyspepsia. TBA-active products concentration in plasma was $5,16 \pm 0,12$ micromole/l, in control group – $3,92 \pm 0,06$ micromole/l. Plasma antioxidant activity of sick animals was reduced ($21,2 \pm 0,06$ %), in control group – $28,6 \pm 0,04$ %. The revealed MDA increase in thrombocytes ($1,66 \pm 0,001$ nmole/ 10^9), and in control group – $0,89 \pm 0,02$ nmole/ 10^9) was the evidence of free radical oxidation (FRO) caused by the reduction of thrombocyte antioxidant activity. Medium molecules' levels in plasma came to $MM_{280} - 0,52 \pm 0,04$ standard units, $MM_{254} - 0,35 \pm 0,02$ standard units, medium molecules amount in thrombocytes $MM_{280} - 0,065 \pm 0,02$ standard units / 10^9 , $MM_{254} - 0,072 \pm 0,01$ standard units/ 10^9 , which are authentically higher than control indexes.

The calves' treatment with the combination phosphopag, acos and calcium gluconate had positive effect on plasma and thrombocyte lipid peroxidation. TBA-active products amount in plasma reduced ($P < 0,01$). By the 10th day of treatment their concentration was $3,97 \pm 0,05$ micromole/l. Lipid peroxidation products reduce lead to normalization of plasma MM_{280} up to $0,32 \pm 0,09$ standard units, $MM_{254} - 0,23 \pm 0,06$ standard units. Lipid peroxidation products reduction in plasma was accompanied by the reduction of MDA basal level in thrombocytes after the 10th day of treatment

($0,90 \pm 0,03$ nmole/ 10^9). The calves' treatment with the combination of phosphopag, acos and calcium gluconate lead to normalization of MM level in trombocytes $MM_{280} - 0,050 \pm 0,06$ standard unit/ 10^9 , $MM_{254} - 0,054 \pm 0,01$ standard unit/ 10^9 .

Thrombocyte content in the sick calves' blood was normal before and after treatment. TA increase was revealed in calves with dyspepsia before treatment especially under the influence of collagen ($20,3 \pm 0,05$ c). TA developed slower in sick calves under the influence of ADP ($36,0 \pm 0,10$ c) and Ristomycin ($31,6 \pm 0,02$ c). Thrombin ($43,6 \pm 0,22$ c) and adrenalin ($82,0 \pm 0,03$ c) TA were revealed later on but developed quicker than in control group ($P < 0,01$). The time of TA development under the influence of the inductors' combination treatment was also accelerated (ADP+adrenalin – $22,0 \pm 0,05$ s, ADP+collagen – $20,0 \pm 0,01$ s, adrenalin+collagen – $19,0 \pm 0,02$ s).

On prescribing the combination of phosphopag, acos and calcium gluconate TA time increased under the influence of all inductors. Collagen turned out to be the most active TA inductor by the 10th day of treatment ($31,0 \pm 0,05$ s). ADP ($39,0 \pm 0,05$ s), Ristomycin ($41,0 \pm 0,12$ s) were less active. TA developed still slower under the influence of thrombin and adrenalin. On the inductors combination TA time increased (ADP+adrenalin – $36,0 \pm 0,03$ s, ADP+collagen – $27,0 \pm 0,05$ s, adrenalin+collagen – $30,0 \pm 0,05$ s).

Thrombocyte intravascular activity in calves with dyspepsia was characterized by its' increase. Discocyte level in sick calves' blood was $62,3 \pm 0,06$ % (in control group – $82,0 \pm 0,16$ %). Disco-echinocyte content has increased 1,60 times. The amount of spherocytes and sphero-echinocytes was also bigger than control data ($13,2 \pm 0,04$ % and $6,8 \pm 0,05$ %, correspondently). The total thrombocyte active forms amount ($37,7 \pm 0,02$ %) in sick calves has increased 2,09 times. The large and small aggregates content in the blood was 4,5 and 45,8 times higher

correspondently than in control group, and thrombocyte amount in sick calves' aggregates was 2,8 times higher than in control group.

Phosphopag, acos and calcium gluconate combined treatment for calves with dyspepsia has allowed to achieve positive ITA dynamics. Real ITA indicators improvement was revealed by the 10th day of treatment. Since phosphopag, acos and calcium gluconate combined treatment discoid thrombocytes content in sick calves blood has increased up to $82,3 \pm 0,6$ %. As a result of treatment the disco-echinocyte, spherocyte and sphero-echinocyte levels in sick animals blood really decreased ($9,4 \pm 0,01$ %, $4,6 \pm 0,05$ % and $2,7 \pm 0,3$ %, correspondently). Since phosphopag, acos and calcium gluconate combined treatment the total amount of thrombocyte active forms has become close to that in control group ($17,7 \pm 0,04$ %). The large and small aggregates amount by the 10th day of treatment has decreased 4,37 and 36,6 times correspondently. Thrombocytes amount in the aggregates has become $5,0 \pm 0,09$ %.

Discussion.

Increased lipid peroxidation in plasma and thrombocytes of calves with dyspepsia reveals the reduction of their organisms' antioxidant system [1] and causes MM level increase in plasma and thrombocytes. Lipid peroxidation normalization and antioxidant plasma potential on MM reduction as a result of the treatment reveals the normalizing effect of phosphopag, acos and calcium gluconate combined treatment on new-born calves with dyspepsia homeostasis. This is the result of all preparations' effect on metabolism and the increase of the organism antioxidant system enzymes expression.

The estimated homeostasis indicators normalization on phosphopag, acos and calcium gluconate combined treatment reveals its' positive effect on thrombocyte hemostasis realization mechanisms in new-born calves with dyspepsia. This is no doubt explained by the metabolism processes

improvement, the reduction of lipid peroxidation and MM toxic effect in plasma and thrombocytes along with the optimization of the process of exogenous signals reception by the thrombocytes. Thrombocyte aggregation activity corresponded to that in control group in new-born calves with dyspepsia being treated with phosphopag, acos and calcium gluconate during 10 days.

Normalization of TA duration in new-born calves under the influence of Ristomycin, and being treated with phosphopag, acos and calcium gluconate, is the evidence of optimization of concentration in blood adhesive molecule i.e. von Willebrand Factor.

Total ITA normalization enables to minimize the thrombocyte complications risk in new-born calves with dyspepsia being treated with phosphopag, acos and calcium gluconate. High effectiveness of the treatment carried out in thrombocyte hemostasis correction in new-born calves with dyspepsia enables to recommend the preparations' combination under research to be widely adopted in live farming.

Literature

1. Volchegorskiy I.A., Dolgushin I.I., Kolesnikov O.L., Tseilikman V.E. Experimental modeling and laboratory research of organism adaptive reactions. Tchelyabinsk, 2000.
2. Gabrielyan N.I., Lipatova V.I. and others. Screening method of middle molecules reveal in biological fluids. Guidelines. M.1985.
3. Kubatiev A.A., Andreev S.V. Lipid peroxidation and thrombosis. // Bull. experim. biol. and medicine 1979.-№ 5.-p. 414-417.
4. Naumov M.M., Medvedev I.N., Efimov K.M. and others. Practical recommendation on "Biopag-D" application in veterinary. Moscow, 2006.
5. Shitikova A.S., Tarkovskaya L.R., Kargin V.D. Method of intravascular

thrombocyte activation reveal and its' importance in clinical practice. // *Clinical and laboratory diagnostics*. 1997.-№ 2.-p. 23-35.

6. Shitikova A.S. Visual micromethod of thrombocyte aggregation research. From the book *Hemostasis. Hemorrhagic diseases main types' physiological mechanisms and diagnostics principles*. Edited by N.N.

Petrischev, L.P. Papayan. Saint-Petersburg, 1999.-p.49-53.

7. Schmith J.B., Ingerman C.M., Silver M.J. Malondialdehyde formation as an indicator of prostaglandin production by human platelet. // *J.Lab. Clin. Med.* 1976.-Vol. 88 (1).-p.167-172.