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AMBER THERAPY EFFECT ON THE INFLAMMATORY PROCESS SEVERITY IN PURULENT WOUNDS IN DOGS

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Abstract. Studying the amount of fibrinogen in the blood plasma of dogs is essential in determining the severity of the inflammatory process. In this regard, the aim of this work was to establish the level of fibrinogen in animals with the purulent-inflammatory process. The studies were performed by spectrophotometric method according to the generally accepted method. During the studies, the content of fibrinogen in the blood plasma of clinically healthy dogs was determined before medical care and during the wound process on the 3rd, 7th, 10th and 14th days of treatment. The results of the study on dogs with purulent skin wounds revel that the amount of fibrinogen before treatment was 2 times higher ($p<0.001$) compared with clinically healthy animals. On the 3rd day of the study, the content of fibrinogen in blood plasma in the animals of experimental group 1 and experimental group 2 was, respectively, 1.6 ($p<0.001$) and 1.5 ($p<0.001$) times higher than in the clinically healthy animals; the amount of fibrinogen was 1.7 times ($p<0.001$) higher in the control group of animals than in the clinically healthy dogs. On the 7th day of treatment, the level of fibrinogen in animals of the experimental group 1 was 1.2 times ($p<0,01$) higher than in clinically healthy dogs, while in the experimental group 2 its content was 1.1 times ($p<0.05$) higher. In contrast, it was 1.4 times ($p<0.001$) higher the control group dogs than in clinically healthy animals. Studies have shown that the administration of succinic acid and intravenous 1.5% solution of reamberin, a drug based on succinic acid, restores the level of fibrinogen in the plasma of dogs with purulent wounds on the 10th day of the wound process, compared with intravenous introduction of 5% glucose solution. The best therapeutic effect was obtained in the group of animals treated with 1.5% solution of reamberin

Keywords: succinic acid, 1.5% reamberin solution, 5% glucose solution, purulent wound, fibrinogen

ВПЛИВ БУРШТИНОТЕРАПІЇ НА ГОСТРОТУ ЗАПАЛЬНОГО ПРОЦЕСУ ЗА ГНІЙНИХ РАН У СОБАК

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Анотація. Для визначення показника гостроти запального процесу актуальним є дослідження кількості фібриногену у плазмі крові собак. У зв'язку з цим метою проведеної роботи було встановлення рівня фібриногену у тварин за гнійно-запального процесу. Дослідження проводились спектрофотометричним методом за загально-прийнятою методикою. Під час досліджень визначено вміст фібриногену у плазмі крові клінічно здорових собак, до надання лікарської допомоги та протягом перебігу ранового процесу на 3-, 7-, 10- та 14-ту доби лікування. За результатами досліджень авторів собак із гнійними ранами шкіри до лікування встановлено, що кількість фібриногену була вища у 2 рази ($p<0,001$) порівняно з клінічно здоровими тваринами. На 3-ю добу досліджень, у тварин I та II дослідних груп вміст фібриногену в плазмі крові був у 1,6 ($p<0,001$) та 1,5 ($p<0,001$) рази відповідно вищим за показники клінічно здорових. У контрольній групі тварин кількість фібриногену була в 1,7 раза ($p<0,001$) вища за показник клінічно здорових собак. На 7-му добу лікування рівень фібриногену у тварин I дослідної групи був у 1,2 раза ($p<0,01$) більшим, порівняно із показником клінічно здорових, водночас у тварин II дослідної групи його вміст був у 1,1 раза ($p<0,05$) вищим. Натомість у собак контрольної групи він був у 1,4 раза ($p<0,001$) вищим за показник у клінічно здорових. За результатами досліджень встановлено, що задавання бурштинової кислоти та внутрішньовенне введення 1,5 %-ного розчину реамберину, препарату на основі бурштинової кислоти, відновлює рівень фібриногену в плазмі крові собак із гнійними ранами вже на 10-ту добу перебігу ранового процесу, порівняно з внутрішньовенним введенням 5 %-ного розчину глюкози. Кращий терапевтичний ефект було одержано у групі тварин, яким застосовували 1,5 %-ний розчин реамберину

Ключові слова: бурштинова кислота, 1,5 %-ний розчин реамберину, 5 %-ний розчин глюкози, гнійна рана, фібриноген

INTRODUCTION

Hemostasis system plays a crucial role in any inflammatory process since it is involved in both in hemostasis and in ensuring the processes of exudation, regeneration and epithelialization. During an injury, arteria get broken and fibrinogen partially passes into the extracellular space and falls out in the form of fibrin threads. The amount of fibrinogen indicates the severity and complexity of the inflammatory process [1]. Fibrinogen and fibrin affect the processes of blood coagulation, fibrinolysis, cellular and matrix interactions, inflammation, wound healing, angiogenesis, neoplasia etc. The result of wound healing largely depends on the structure of fibrin, its fibers thickness, the number of branching points, porosity and permeability [2]. Binding of fibrin (hegen) to hemostasis proteins and platelets, as well as to some cells, such as endothelial cells, smooth muscle cells, fibroblasts, leukocytes, and keratinocytes, is a necessary element in the wound healing process [3].

The authors [4] claim that stimulants of wound healing are becoming widespread in wounds treatment in small pets along with conservative and surgical treatment. The stimulants include sugar, bee honey, a complex compound of copper and tripeptide, a derivative of the carbohydrate mannose – acemannan, D-glucose polysaccharide, platelet derivatives, as well as chitosan derived from the exoskeleton of mollusks.

Inflammatory process is a reaction of the body to various types of damage, which is aimed at detecting and neutralizing the traumatic factor, enzymatic melting of non-viable tissues, protective barrier formation and, if necessary, covering the affected tissue [5]. A lot of changes, including biochemical ones, take place in the animal's body during the wound process. Scientists have been searching for new effective non-toxic substances

having a wide range of effects on certain changes and the body as a whole in order to correct these changes. Succinic acid and its medications are among these substances.

Succinic acid and drugs based on it have been used more and more widely in scientific experiments conducted in various industries, medicine, veterinary medicine [6-9]. Tissue hypoxia with the development of mitochondrial dysfunction occurs in most intracellular pathological processes, including the liver, which justifies the optimal way to correct cytotoxic changes by introducing additional substrates of energy metabolism. In particular, the introduction of exogenous succinate – a natural metabolite of the Krebs cycle – promotes normal aerobic oxidation through eliminating the oxidative phosphorylation separation and microsomal processes inhibition [10].

Succinic acid and its salts (succinates) are a universal intracellular metabolite involved in the body metabolic reactions. The importance of succinic acid in cell metabolism is due to its participation in the tricarboxylic acid cycle (TAC, Krebs cycle) and oxidative phosphorylation processes. Oxidation of succinic acid in the Krebs cycle is carried out using a specific enzyme – succinate dehydrogenase, localized on the inner surface of the inner membrane of mitochondria. Its activity does not depend on the ratio of oxidized and restored forms of Nicotinamide adenine dinucleotide ($NADH/NAD^+$) and this characteristic feature provides saving the energy synthesizing function of mitochondria under hypoxia in NAD-dependent cell respiration disorders [11].

Development and search for effective preparations for the prevention and treatment of disorders in metabolism, correction of biochemical parameters and increase in the natural resistance of the organisms of animals is a relevant problem. Metabolic correction is a direction in therapy which is based on use of preparations of natural origin which combine efficiency and safety and impose no pharmacological load due to their bioavailability. These requirements are met by natural metabolites of the Krebs cycle, particularly com-pounds of succinic acid. Succinic acid is a universal intracellular metabolite with a broad range of action towards the parameters of vitality of a living organism. The system that uses succinic acid for producing energy, by its power, becomes significantly superior to the other systems of organisms' energy production. Therefore, succinic acid provides a broad range of non-specific treatment effects based on the influence on the process of tissue metabolism: cellular respiration, ionic transport, and synthesis of proteins [12].

Since during trauma and purulent-inflammatory processes, the amount of fibrinogen in the blood plasma increases due to the enhancement of biosynthesis by hepatocytes, it will be appropriate to determine its content as an indicator of the severity of the inflammatory process.

MATERIALS AND METHODS

To collect data for the research, author studied clinically healthy dogs and the ones with purulent wounds. The animals were taken to the surgical clinic of the Faculty of Veterinary Medicine of Bila Tserkva National Agrarian University for their clinical examination and treatment. The animals were 2 to 5 years old, weighing 10-15 kg, clinically healthy and with purulent wounds of the skin and soft tissues. The research groups of animals were formed on the principle of par-analogues. Research blood sampling was performed in dogs during a clinical examination of healthy animals as well as before treatment, on the 3^{rd}, 7^{th}, 10^{th} and 14^{th} day of therapy in animals with purulent wounds. Sample blood amounted 3-3.5 ml taken from the subcutaneous vein of the forearm or saphenous vein. Blood plasma samples were frozen at a temperature of 20^oC.

Local treatment of purulent wounds in animals involved primary surgical treatment, sanitation with solutions of 3% hydrogen peroxide and 0.5% chlorhexidine in the amount of 100 ml each and passive drainage with Levomekol ointment twice a day. The number of days of wound drainage depended on the rate of their purification from purulent exudate. Dogs of the I experimental group (n = 10) were given succinic acid at a dose of 0.1 g/kg of body weight. Animals of experimental group 2 (n = 10) were intravenously administered 1.5% solution of reamberin at a dose of 10 ml/kg of body weight. Dogs of the control group (n = 10) were intravenously injected with 5% glucose solution at a dose of 10 ml/kg of body weight. The animals were treated for 5 days.

Fibrinogen concentration in blood plasma was determined by the method of V.O. Belitzer et al. [13]. The method is based on the enzymatic conversion of fibrinogen to fibrin with thrombin in the presence of calcium ions and monoiodoacetic

acid, which prevents the fibrin stabilization by factor XIII. Calcium ions promote the rapid and complete conversion of fibrinogen into fibrin, and monoiodoacetic acid contributes to elimination of some whey proteins retention in the clot and facilitates its dissolution. Reagents and materials: phosphate buffer / 0.06M / pH 7.0; 0.15 M sodium chloride solution (pure); 0.04 M monoiodoacetic acid solution; thrombin (1000 units/mg). Equipment: thermostat, spectrophotometer (SF-46).

Methodology. 0.2 ml of plasma is added 1.6 ml of 0.06 M phosphate buffer. The mixture is placed in a thermostat at 37°C. In 1-2 minutes, 0.1 ml of a 0.04 M solution of monoiodoacetic acid is added to the mixture. 0.1 ml of thrombin is added in 3 min. Under using calcium-free thrombin preparations, 0.1 ml of 0.02 M calcium chloride is added to the sample before thrombin addition. The substance is stirred thoroughly with a stick and incubated for minimum of 15 minutes at 37°C. The clot is wound on a stick, washed twice with water and 0.15 M sodium chloride solution and dried. The clot is dissolved in 5 ml of 1.5% acetic acid solution and the optical density is determined with a spectrophotometer at 2 wavelengths: 280 and 320 nm. Extinction is stable. Control – 1.5% acetic acid.

Calculations are performed according to the formula (1):

$$X = \frac{\Delta E \times 255}{15.067} \quad (1)$$

where X is fibrinogen concentration g/l; ΔE is the difference between the values of extinctions obtained at 280 and 320 nm; 255 – coefficient for presenting the amount of fibrinogen in g/l in the analysis carried out in 0.2 ml of plasma, taking into account the correction for its determination by the actual analysis of the fibrin solution; 15.067 – extinction coefficient for fibrinogen in an acidic medium at a wavelength of 280 nm.

RESULTS AND DISCUSSION

The results of the study on dogs with purulent skin wounds indicated that the amount of fibrinogen before treatment (Fig. 1) was 2 times higher ($p<0.001$) compared to clinically healthy animals (2.61 ± 0.08 g/l) and made 5.26 ± 0.16 g/l. Activation of blood clotting occurs immediately after the tissue injury. In this case, soluble fibrinogen is converted into insoluble fibrin. In addition to its hemostatic function, a layer of fibrin on the damaged surface and fibrin clots in the lumens of blood vessels contribute to the localization of the inflammatory nidus. Such barriers trap microorganisms, mechanically preventing their dissemination [14].

On the 3rd day of the study, the content of fibrinogen in blood plasma in the animals of experimental group 1 (4.06 ± 0.14 g/l) and experimental group 2 (3.91 ± 0.12 g/l) was, respectively, 1.6 ($p<0.001$) and 1.5 ($p<0.001$) times higher than in the clinically healthy animals; the amount of fibrinogen was 1.7 times ($p<0.001$) higher in the control group of animals (4.53 ± 0.14 g/l) r than in the clinically healthy dogs. The level of fibrinogen was significantly ($p<0.05$) higher in the control group dogs compared to that in the animals of the experimental group 1; it was 1.2 times ($p<0.01$) higher compared to the fibrinogen level in the animals of experimental group 2. Fibrinogen content on the 3rd day of treatment was 1.7 times higher ($p<0.001$) in the control group compared with clinically healthy animals.

On the 7th day of treatment, the level of fibrinogen in animals of the experimental group 1 was 1.2 times ($p<0.01$) higher than in clinically healthy dogs, while in the experimental group 2 its content was 1.1 times ($p<0.05$) higher. In contrast, it was 1.4 times ($p<0.001$) higher in the control group dogs than in clinically healthy animals and 1.2 ($p<0.01$) lower than in animals of experimental groups 1 and 2.

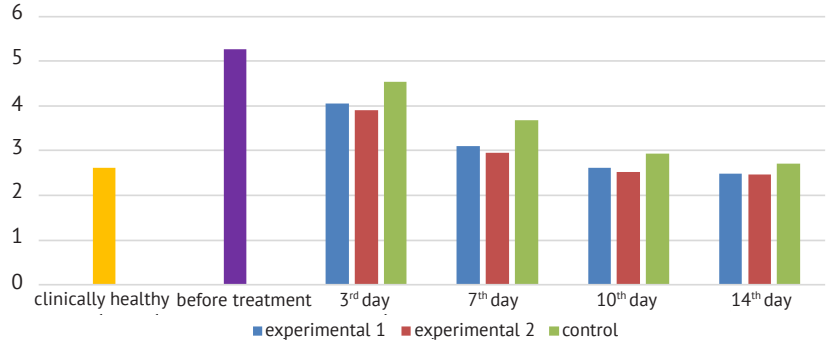

Figure 1. *Dynamics of fibrinogen in dogs with purulent wounds, g/l*

Medications based on succinic acid quickly normalize the content of fibrinogen in the blood plasma of animals with purulent wounds due to their membrane-stabilizing action. Therefore, as early as on the 10th day of the course of the wound process in dogs of the 1 and 2 experimental groups, who received amber therapy, the amount of fibrinogen in the blood plasma decreased to the level of clinically healthy and later, until the 14th day of treatment, remained within the normal range. In the control group animals treated with 5% glucose solution, the fibrinogen content gradually decreased, but it remained 1.1 times higher ($p<0.05$) than in clinically healthy animals and those of the experimental group 1, and 1.2 times ($p<0,01$) higher than the indicator in the experimental group 2 animals.

On the 14th day of treatment, it was found that in the dogs of the control group, the fibrinogen content (2.71 ± 0.09 g/l) was at the level of the indicator of clinically healthy dogs, but still 1.1 times higher than the indicator in animals of the 2 experimental group (2.46 ± 0.09 g/l).

Medications based on succinic acid have improved pharmacological properties with the greatest possible reduction of possible side effects [15]. The research established that the use of amber therapy, namely oral administering of succinic acid and intravenous administering of 1.5% solution of reamberin contributed to a faster recovery of fibrinogen content compared to the control group of animals treated with intravenous 5% glucose solution.

CONCLUSIONS

The hemostasis system is essential in successful regeneration of injured tissues. Fibrinolytic link is one of the regeneration components promoting the regenerative processes. It is fibrin that enters the wound cavity after vessels injure and increases their porosity, forms the primary wound adhesion, which later germinates with capillaries and forms a dense vascular network. Therefore, determining the amount of fibrinogen in the dogs' blood under the purulent-inflammatory processes is an important diagnostic indicator enabling to determine the intensity of the wound process and predict its duration. Fibrinogen content was restored on the 10th day under feeding succinic acid to the dogs with purulent wounds. This reduced the duration of treatment by 1.2 ($p<0.001$) times compared with the control group. Intravenous reamberin treatment provided fibrinogen level recovery in the blood of dogs on the 7th day of treatment which resulted in 1.3 ($p<0.001$) times faster wound healing compared to the control group. The study has found out that the use of succinic acid and medications based on it contributes to faster normalization of fibrinogen content in the blood plasma of dogs, increase the body resistance and reduce the time of purulent wounds healing. Prospects for further research. Conducting research on the use of succinic acid and medications based on it for treating other surgical pathology diseases.

REFERENCES

[1] Pidborska, R.V. (2010). The effect of ozonated 0.87% NaCL solution on the content of fibrinogen in the blood of dogs with purulent wounds. *Bulletin of the Poltava State Agrarian Academy*, 4, 204-206.

[2] Rublenko, M.V., Khanyeyev, V., & Rukhlyada, V. (2004). The haemostatic system changes in dogs with purulent wounds, depending on microorganisms species in pus and treatment method. *Veterinary Medicine of Ukraine,* 6, 38-40.

[3] Laurens, N., Koolwijk, P., & de Maat, M.P.M. (2006). Fibrin structure and wound healing. *Journal of Thrombosis and Haemostasis,* 4(5), 932-939. doi: 10.1111/j.1538-7836.2006.01861.x.

[4] Sweim, S., & Boling, M. (2015). Some aspects of wound healing in small pets. *Veterinary Practice*, 11, 4-14.

[5] Hierdieva, A.O., & Ilnitsky, M.H. (2017). The use of amber therapy in the treatment of dogs with purulent wounds. In *"Veterinary support of intensive technologies in animal husbandry, food safety and quality": Materials of the international scientific-practical conference* (pp. 3-4). Bila Tserkva: Bila Tserkva National Agrarian University.

[6] Cancelas, J., Villanueva-Penacarrillo, M.L., Valverde, I., & Malaisse, W.J. (2001). Synergistic insulinotropic effects of succinic acid dimethyl ester and exendin-4 in anaesthetized rats. *International Journal or Molecular Medicine,* 8(3), 269-271. doi: 10.3892/ijmm.8.3.269.

[7] Juliet Sheela, K., Subbulakshmi, N., & Subramanian, P. (2017). Local symmetry and location of VO2+ ion in potassium succinate-succinic acid single crystal through EPR studies. *AIP Conference Proceedings*, 1832, article number 100006. doi: 10.1063/1.4980608.

[8] Ladrière, L., & Malaisse, W.J. (2000). Effects of the dimethyl ester on succinic acid on the hormonal and metabolic response to exercise in hereditarily diabetic starved rats. *Cell Biochemistry and Function*, 18(3), 153-160. doi: 10.1002/1099-0844(200009)18:3<153::AID-CBF858>3.0.CO;2-Q

[9] Simonova, N., Dorovskikh, V., Kropotov, A., Kotelnicova, M., Sharberg, M., Maysak, A., Chernysheva, A., & Kabar, M. (2018). Comparative effectiveness of succinic acid and reamberin in the oxidative stress in experiment. *Bulletin Physiology and Pathology of Respiration*, 1(70), 78-82. doi: 10.12737/article_5c126def73b749.24896834.

[10] Sukhanov, D.S., Pavlova, M.V., & Vinogradova, G.N. (2013). Clinical efficiency of succinic acid-based infusion solutions in the treatment of liver damage caused by anti-TB drugs. *Tuberculosis and Lung Diseases*, 8, 50-56.

[11] Smirnov, A.V., Nesterova, O.B., & Golubev, R.V. (2014). Succinic acid and its application in medicine. Succinic acid: A metabolite and regulator of metabolism in humans. *Nephrology*, 18(2), 33-41.

[12] Lieshchova, M.A., Bilan, M.V., Bohomaz, A.A., Tishkina, N.M., & Brygadyrenko, V.V. (2020). Effect of succinic acid on the organism of mice and their intestinal microbiota against the background of excessive fat consumption. *Regulatory Mechanisms in Biosystems*, 11(2), 153-161. doi: 10.15421/022023.

[13] Belitzer, V.O., Varetska, T.V., & Veremeenko, K.M. (1997). Quantitative determination of fibrinogen in human blood plasma. *Laboratory Diagnostics,* 2, 52-55.

[14] Khaneev, V.V. (2004). *Hemostasis and its correction in surgical infection in dogs* (Doctoral dissertation, Bila Tserkva National Agrarian University, Bila Tserkva, Ukraine).

[15] Manohar, R., Kutumbarao, N., & Nagampalli, R. (2018). Structural insights and binding of a natural ligand, succinic acid with serine and cysteine proteases. *Biochemical and Biophysical Research Communications*, 495(1), 679-685. doi: 10.1016/j.bbrc.2017.11.033.