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Hematological and Immunological Blood Parameters in the Treatment of Infected Wounds in Dogs

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Abstract: In recent years, multicomponent pharmacological mixtures that actively penetrate the depth of the wound and have an antimicrobial effect are widely used to treat wounds of various etiologies. The composition of tested multicomponent ointments includes polyethylene oxides, anesthetics, pyrimidine derivatives and others. Nevertheless, in most cases existing drugs are not aimed at specific phases of healing and they are effective with regard to a wide range of microorganisms. This study demonstrated materials on the study of the morphological and immunological research of the blood composition of dogs which received pharmacological mixtures developed by us (mixture of metsin and actovin liniment). In the I phase of wound healing, we used the multicomponent mixture "metsin" which is aimed at cleansing the wound from damaged tissues as well as having an anesthetic effect, reducing swelling and antimicrobial effect. In the II-III phase of wound healing, we used the "actovin" liniment which improves the blood supply to the tissues, stimulates wound healing and the elimination of repeated microbial seeding.

Key words: Hematological studies, erythrocyte sedimentation rate, leukocytes, immunological status, multicomponent mixtures, microorganisms

INTRODUCTION

Blood is the liquid tissue of the organism, consisting of plasma and blood cells. Erythrocytes, leukocytes and platelets are formed elements of blood. Blood, lymph and tissue fluid play an important role in metabolism of animals (Aburjai *et al*., 2019; Anima *et al*., 2019). According to some researchers (Devi *et al*., 2018; Dillekas and Straume, 2018), blood is a "reflection" of the state of the organism. The morphological composition of blood is one of the main indicators of the physiological state of the body and allows to more fully judge the course of metabolic processes in it. The blood plasma contains proteins, lipids, glucose, lactic and pyruvic acid, non-protein nitrogenous substances, mineral salts, enzymes, hormones, vitamins and pigments. Blood stream transports oxygen, carbon dioxide and nitrogen. Homeostasis is maintained with optimal functioning of the organs and tissues of the body (Elgohary *et al*., 2018).

One of the most important diagnostic methods is the morphological study of blood which most fully presents the reaction of all internal organs under the influence of various pathological factors on the body (Frees, 2018). The main blood parameters show the state of the body and make it possible to judge its protective abilities as the processes taking place in the body always affect the morphological and protein composition of the blood (Kiya and Kubo, 2019). Besides, erythrocyte sedimentation rate is determined along with the study of the morphological composition of the blood. This indicator plays an important role in the diagnosis of pathologies which increases during inflammatory processes in the animal's body in particular during the wound process (Lima *et al*., 2018; Miguel *et al*., 2019). As a result of a number of studies, it has been established that the occurrence and rate of infection on the wound surface depends on both the pathogenic properties of the microflora and local factors of immune defense

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(Sami *et al.*, 2019). Weakening of the immune system of the body plays an important role in the development of wound infection. This is primarily due to the fact that the animal body is exposed to many unfavorable external and internal factors connected with poor conditions of housing and feeding (Tsiouris and Tsiouri, 2017; Delft *et al.*, 2019).

MATERIALS AND METHODS

Studies were conducted at the Department of Non-communicable Diseases of the Kazan State Academy of Veterinary Medicine. Blood was taken from the lateral vein of the lower leg of the dogs before providing medical assistance for hematological studies, then on the 3rd, 7th 14th and 21st day of therapy. Morphological blood tests were conducted according to the following parameters: Erythrocyte Sedimentation Rate (ESR), Red Blood Cell count (RBC), White Blood Count (WBC) and Hemoglobin (HGB). The analysis was carried out on an automatic hematological analyzer for veterinary medicine BC-2800 Vet, Mindray. ESR was determined by the TP Panchenkov's method.

Immunological studies were carried out on 3 indicators: phagocytic activity, phagocytic number, phagocytic index. The level of immunological parameters was established by the use of a light microscope "Olympus-CX 31" and latex for phagocytosis in a 10% suspension. Mathematical studies of the data were conducted using the program BioSta 2009. Statistical significance between the control and experimental groups was assessed by the student criterion. 15 not pedigree dogs with a live weight of 10-13 kg and aged 2-5 years were selected to simulate infected wounds. Then 3 groups of animals were formed: control, experimental group No. 1 and experimental group No. 2. Rules for preclinical studies of drugs were observed in animal studies. The dogs were chosen according to the type of analogues and were in the same conditions of the vivarium. General and local anesthesias were used before the modeling wounds in all experimental groups.

First of all, general anesthesia was used. Then, skin with the underlying tissue was pulled in the form of a cone of a height of 1 cm through a hole of a metal plate with a diameter of 3.0 cm. The fragment was cut out. It was made in order to create wounds in selected animals on the lateral surface of the thigh. Further, a gauze plug moistened in a 70% acetic acid solution was applied to the formed wound surface for 5 sec. Afterwards, daily culture of *Staphylococcus aureus* with a suspension of cattle feces was applied to the affected surface in order to infect the wound the next day.

Starting from the 3 day after modeling and infection contamination of wounds, infected wounds of the animals of the control group were treated daily twice with Levomekol ointment; wounds of the dogs of the second group (experimental group No. 1) were treated with an alcohol solution of aloe. In the first phase of healing according to M.I. Kuzin, wounds of the dogs of the third group (experimental group No. 2) were treated with the help of the drug "metsin". In the treatment of animals in the second and third phases of healing according to M.I. Kuzin, "actovin" was used in order to prolong the drug's action and prevent microbial recontamination of an infected wound.

RESULTS AND DISCUSSION

All 3 groups of animals showed pronounced signs of inflammation: hyperemia, local temperature increase, soreness in the first days of wound modeling. Changes in the morphological parameters of blood were established in the course of therapy in animals. Table 1 presents a

Table 1: Morphological blood parameters in dogs

Parameters/Group	Duration of study, days				
	Before treatment	3	7	14	21
Erythrocytes (10^{12}/l)					
Control	5.62±0.31	6.,98±0.3	6.72±0.3	6.96±0.7	6.4±0.1
1-experimental	5.92±0.2	6.64±0.3	6.74±0.5	6.74±0.3	6.5±0.25
2-experimental	6.1±0.1	6.68±0.2[1]	6.8±0.3	7.28±0.3	5.9±0.3
Leukocytes (10^9/l)					
Control	8.12±0.6	12.42±1.9	12.58±0.6	11.4±0.4	10.14±0.2
1-experimental	8.52±0.4	13.56±1.8	12.32±0.6	10.7±0.5	9.5±0.4
2-experimental	7.96±0.4	11.58±0.8	9.64±0.6[1,2]	9.3±0.3	8.01±0.5
Hemoglobin (g/L)					
Control	159.22±7.5	165.26±3.4	179.28±5.2	183.3±4.9	152.36±1.5
1-experimental	152.92±5.04	169.74±3.3	180.16±6.7	183.08±5.9	148.25±1.2
2-experimental	157.84±6.8	174.88±4.3	178.08±4.4	176.02±7.7	159.4±0.7
ESR (mm/h)					
Control	2.85±0.6	11.6±0.7	7.88±1.0	7.64±0.3	6.8±0.3
1-experimental	4.83±1.01	12.2±0.8	8.36±0.6[1]	8.10±0.4	7.05±0.4
2-experimental	3.5±1.0	12.1±0.5	8.56±0.1[1]	7.21±0.4	4.1±0.4

[1, 2]: are significent values

hematic picture of all the studied groups conducted before the start of the experiment as well as on the 3rd, 7th, 14th and 21st day. There was a decrease in the content of leukocytes from the 7th day to the 14th day which is connected with the extinction of the inflammatory process in the pathological focus.

By the 3rd day, the number of leukocytes in the animals of the control group was 12.42±1.9×10^9/l in the experimental group No. 1 -13.56±1.8×10^9/l in the experimental group No. 2 -11.58±0, 8×10^9/l which indicates an increase in indicators of the relative average standard value by 52, 60 and 45%, respectively. This fact is a consequence of the development of the inflammatory process.

A slight decrease in the number of leukocytes in the control group and experimental group No. 1 was found by the 21st day of therapy. In the control group and experimental group No. 1, the number of white blood cells decreased by 11 and 24%, respectively.

The number of leukocytes was 8.01±0.5×10^9/l in the experimental group No. 2 by the 21st day which indicates the alleviation of the state of the animals, indicating the effectiveness of the drugs. Parameters of ESR indicate that all groups had an inflammatory process by the 3rd day of treatment.

In the control group it is 11.6 mm/h in the experimental group No. 1 -12.2 mm/h and in the experimental group No. 2 -12.1 mm/h which is 3.3; 3.48; 3.45 higher than the average standard value, respectively and is connected with an acute reaction of the body to the inflammatory process. By the 7th day, the ESR parameters were extremely above the norm, indicating an ongoing inflammatory process. The ESR parameter was 7.88 mm/h in the control group in the experimental group No. 1 -8.36 mm/h and in the experimental group No. 2 -8.56 mm/h, which was 2.25, 2.4, 2.44 higher than normal, respectively. By the 21st day of treatment, the ESR indicator decreased to 6.8 mm/h in the control group, it decreased to 7.05 mm/h in the experimental group No. 1 and the indicator was 4.1 mm/h in the experimental group No. 2 which was 1.9, 2, 1.17 above the average standard value, respectively, indicating the extinction of the inflammatory process.

Thus, it was revealed that all experimental groups by the 3rd day of treatment had an acute reaction to the inflammatory process in the body. The process of normalization of ESR parameters proceeded most intensively in the experimental group No. 2 compared with other experimental groups. There was a decrease in the number of leukocytes in the shortest possible time in the experimental group No. 2 after the use of drugs which indicates an effective anti-inflammatory action of the drugs that, we have developed. There was a decrease in the erythrocyte indices of 5.9±0.3×1012/l in animals that received "metsin" and "actin" (experimental group No. 2) compared to the average standard indicator of 6.1×1012/l by the end of the experimental study by the 21st day. It was revealed in the control and the 1st experimental groups that the indicators were 6.4±0.1×1012/l and 6.5±0.25×1012/l which is higher by 9 and 13% of the average standard values, respectively. This dynamics is most likely connected with a decrease in blood viscosity and is the result of rehydration of the body as a sign of recovery. The hemoglobin in all tested animals was slightly increased but it was within the reference ranges (hemoglobin: 120-180 g/L for this specimen of animal) which is connected with an increase in the respiratory function of the blood. Table 2 shows the changes in cellular immunity factors of all experimental groups of dogs with various methods of treating infected wounds. Analyzing the immunological parameters of the blood of all experimental groups of dogs, shown in Table 2, it was established that the tendency to increase the indicators of phagocytic activity, phagocytic number and phagocytic index is a consequence of an increase in the immune status of the body. By the 7th day of the wound surface treatment, obvious improvement in cellular immunity indices was observed in the experimental group No. 2. This fact proves that the drugs we have developed have a positive effect on the increase in the immune status of the dogs.

Table 2: Changes in cellular immunity factors of all experimental groups of dogs

Indicators	Phagocytic activity (%)	Phagocytic number (cu)	Phagocytic index (cu)
Control group			
Before treatment	28.63±2.45	2.37±2.37	1.34±0.2
By the 3rd day	32.45±1.23	3.9±0.27	1.69±0.08
By the 7th day	36.26±1.22	5.92±0.17	3.89±0.11
Experimental group 1			
Before treatment	26.3±2.4	2.5±0.08	1.23±0.13
By the 3rd day	34.47±1.6	4.11±0.24	1.55±0.07
By the 7th day	38.99±0.7	6.41±0.17	3.76±0.25
Experimental group 2			
Before treatment	23.5±1.4	2.14±0.2	1.26±0.14
By the 3rd day	35.48±1.3	3.59±0.19	1.61±0.2
By the 7th day	31±0.75	4.37±0.08	2.6±0.08

CONCLUSION

As a result of the use of the developed multicomponent mixtures "metsin" and "actovin" for the treatment of infected wounds in dogs with daily double treatment, we found that these drugs have an effective therapeutic action which was confirmed by the results of hematological and immunological studies. In addition, the use of these drugs has a favorable impact on the course of the inflammatory process and contributes to the normalization of patient's blood parameters.

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