

Slusar G.V., postgraduate student

Poltava State Agrarian Academy

DYNAMICS OF IMMUNOLOGIC INDICES BLOOD OF DOGS UNDER VARIOUS METHODS OF TREATMENT OF WOUNDS

Reviewer – candidate of veterinary sciences O. O. Milanko

An dynamics of immunological parameters for the different treatment of wounds in dogs. It is shown that topical preparations of hyaluronic acid and tryfuzolu in treatment of purulent wounds in dogs stimulates healing by accelerating reparative processes. Established faster recovery performance of specific and nonspecific resistance of the organism in a phase of regeneration and proliferation of wound healing: increase of T and B lymphocytes, IRI, the number of phagocytic and NBT-test.

Keywords: *dogs, wounds.*

The process of healing is one of the main mechanisms to protect the body from injury. However, there are circumstances when this physiological process is broken - and wound repair is slow. Therefore, the search for new methods of rational immunosuppressive therapy remains an important problem of modern surgery.

Rate of wound healing depends on the nature of the injury, the number of nonviable tissue and virulence of microorganisms. Indicators of immunological reactivity of the organism - the factors that determine the development of inflammatory processes. However, in the treatment of purulent surgical diseases using antibiotics observed decline in immunity, especially in animals with hiperhichnym type reactivity. Therefore, the use of immunostimulating therapy is a necessary component of pathogenesis of complex wounds treatment [1].

Found that triazole derivatives with immunostimulating, hepatoprotective, anti-inflammatory and antioxidant activity. Drugs stimulate erythro-and leykopoetychni function. In addition, they have bactericidal and fungicidal properties. Use of surgical treatment and intramuscular injections tryfuzolu enables to shorten healing [2].

Purpose - to establish immunological parameters of blood for various treatments of wounds in dogs.

Research carried out on mongrel dogs with skin-muscle wounds area 50-60 cm². The first five days of daily conducted an audit of wounds and local machining, applied course of antibiotic therapy (15% amoksytsyklin, INVESA). Then the animals were divided into two groups. For the treatment of animals of the first group used the ointment Methyluracilum of miramistin and adding it 1% hyaluronic acid and 1% tryfuzol. Ointment used as surface applications per day. The second

group served as control (ointment applied Methyluracilum of miramistin). The animals of both experimental groups were collected blood before the experiment and on the 6th, 12th and 24th day after injury.

Ointment Methyluracilum of miramistin ("Darnitsya", Ukraine) contains Methyluracilum that stimulates metabolism and miramistin - cationic antiseptic. Tryfuzol - derived 1,2,4-triazoles, has antioxidant, hepatoprotective, anti-inflammatory properties. It is recommended for the treatment of localized inflammatory and suppurative processes [2].

Hyaluronic Acid - nesulfatovanyy glycosaminoglycans, which is part of the connective, epithelial, and neural tissues. For studies was used hyaluronic acid bacterial origin (Streptococcus equi) company «Fluka» (Switzerland).

Number of T-and B-lymphocytes was determined by phenotyping in rosette tests with particles coated with monoclonal antibodies. To assess the functional status of B-systems of immunity in serum were determined content of the three major classes of immunoglobulins - M, G, A by radial immunodiffusion in agar. Phagocytic activity was determined by the methods of A. Prylutsko. Oxidation-reduction ability of neutrophils was evaluated in NBT -test [3].

Clinical examination revealed that the use of II and III phases of wound healing ointment Methyluracilum mirimistynom with the addition of hyaluronic acid and tryfuzol noted the accelerating growth of granulation tissue and epithelialization boundary. The animals of the first group healing recorded on 23-25th, the second - to 27-29th day.

Immune status was assessed by the characteristic functional activity of T-and B-lymphocytes with their absolute and relative content in the blood. On the twelfth day studies in animals of the first group significantly increased the number of lymphocytes and was $4344,5 \pm 243,7$ cells / ml, which is higher than the sixth day of the experiment, and 1,8 times higher than the original figure by 64,3 % ($p < 0,01$). The level of T-lymphocytes was $41,83 \pm 1,49\%$, which is higher than the previous figure of 10,1%, besides their absolute number increased to $1816,5 \pm 116,04$ cells / ml, which is twice more than of the sixth era of research.

In the second control group of animals on the twelfth day studies also recorded growth in the number of lymphocytes, but less pronounced than in the first. This figure was $2684,33 \pm 346,79$ cells / ml, which is higher than the previous value by 16,8%, but lower than that of animals of the first group, 1,6 times ($p < 0,05$). Percentage of T lymphocytes was $33,00 \pm 1,15\%$, which is less than that of the subjects in the first group by 21,1% ($p < 0,05$). Content regulatory subpopulations was CD4 + - $29,83 \pm 0,91\%$ and CD8 + lymphocytes - $19,83 \pm 0,91\%$. Immunoregulatory index - $1,51 \pm 0,08$, which is lower than in dogs of the first group, 20,9% ($p < 0,01$).

Absolute and relative lymphocytosis develops after transmission of antigen to lymphocytes from antyhenprezentuyuchyh cells.

Go lymphocytic immune response in phase clinically detected decrease of clinical signs of inflammation - general (hyperthermia, intoxication) and local (swelling, redness, pain). Lymphocyte reaction stimulating factors of natural resistance, resulting in an immune response becomes more efficient. During the development of inflammatory reactions immunoregulatory index reaches high levels by a significant percentage of T-helper cells (CD4 + T cells). During regeneration IRI decreases due to the increase of the level of CD8 + T cells (killer).

In the 24 th day after the start of studies in animals of both groups recorded a declining trend in the content of all subpopulations of lymphocytes. The absolute number of lymphocytes in the first group of dogs was $2807,0 \pm 142,51$ cells / ml, which is lower than the previous value of 1.5 times, but higher than that of animals of the second group, 13,9% ($p < 0,05$). During this period experienced reduced percentage of T lymphocytes - $30,33 \pm 1,36\%$, which is lower by 27,5% than at the twelfth day of research and 25,7% less than the original value. Their absolute number was $849,83 \pm 50,36$ cells / ml, which is 18,3% higher than in animals of the second group. Reduced CD3 + lymphocytes was mainly due to T-helper - this figure was 26,67%, which is less than the previous value by 35,2%. The level of CD8 + lymphocytes also decreased to $18,17 \pm 0,48\%$ compared to the baseline - 27,67% ($p < 0,001$). Immunoregulatory index was $1,47 \pm 0,05$.

In animals, the second control group of 24-th day studies also recorded reduced levels of lymphocytes - $2464,67 \pm 56,01$ cells / ml, which is significantly lower than in the first group of dogs by 13,9% ($p < 0,05$). Percentage of T lymphocytes was $29,17 \pm 2,44\%$, their absolute number - $718,5 \pm 62,97$ cells / ml. There were also changes among subpopulations of CD3 + lymphocytes: the content of T-helper cells decreased to $23,17 \pm 1,14\%$, which is less than the initial index by 52,4% and significantly lower than in animals of the first experimental group at 13,1%. In contrast, the percentage of CD8 + lymphocytes increased and amounted to $20,5 \pm 0,8\%$, which is higher than that of dogs in the first group by 12,8% ($p < 0,05$). Due to this decreased immunoregulatory index - $1,14 \pm 0,06$ and was significantly lower than in animals of the first group. This pattern indicates the likelihood of chronicity of infection due to incomplete eradication of the pathogen.

To quantify the level of cellular immune effector determine the contents of large granular lymphocytes - cell population, which consists of natural killer cells. In both groups of animals likely increase natural killer content recorded on the twelfth day of the experiment. The corresponding figures were in the first group of animals $17,83 \pm 0,95$ cells / ml, in the second - $16,17 \pm 0,87$, which is higher than before the experiment in 1,9 and 1,8-fold ($p < 0,01$ and $p < 0,05$, respectively). Similar changes are also found at the 24 th day of observation.

Effector link humoral immune response is the synthesis of antibodies and phagocytosis, which are neutralized by immune complexes formed. Humoral immunity characterize the level of

CD22 +-cells (B lymphocytes) and levels of different classes of immunoglobulins (IgM, IgG, IgE, serum and secretory IgA).

On the twelfth day of the experiment the animals of the first group of B-lymphocyte content was $26,33 \pm 0,61\%$, which is above the reference value of $8,9\%$. However, their absolute number ($1148,5 \pm 84,12$ cells / ml) compared with the initial value increased 1,8-fold ($p < 0,01$). Percentage of B lymphocytes in the animals of the second group was $24,5 \pm 0,99$, which is below the output by $4,6\%$. The absolute number of CD22 + lymphocytes - $653,5 \pm 79,71$ cells / ml, which is lower than in the animals of the first group, 1,8-fold ($p < 0,05$).

Immunoglobulin M - a period of acute antibody immune response that synthesizes plasma cells at the first contact with a particular pathogen. They have the ability to activate complement, to realize komplementzalezhnoyi cytotoxicity. High concentrations of specific IgM in both groups of dogs were recorded on the twelfth day of research. The animals of the first group - $1,2 \pm 0,02$ g / l, which is higher than before the study, $36,4\%$ ($p < 0,001$) in the second dog - $1,14 \pm 0,02$ g / l, above the original index by $12,9\%$ ($p < 0,05$), but lower than in animals of the first group by 5% . Subsequent studies found reduction of IgM in both groups of animals.

Immunoglobulin G - a late phase antibody immune response, they are more specific than IgM; having only two antigen binding sites and a lower molecular weight, and therefore has a greater permeability to peripheral tissues. Maximum values for 24 th day - $1,12 \pm 0,01$ g / l in the first group and $1,1 \pm 0,02$ g / l - in the second, which is higher than at the beginning of the experiment was $27,3\%$ ($p < 0,001$) and $8,9\%$ ($p < 0,05$), respectively. High levels of specific IgG were recorded during the regenerative process and recovery, insofar as it is this class of antibody-producing cells of the immune memory.

The content of immunoglobulin A in the experiment underwent insignificant fluctuations in animals of both groups: the first group of dogs these figures ranged from $0,9$ to $0,95$ g / l, the second - $0,92$ - $1,01$ g / l.

The system of innate resistance characterize the magnitude of phagocytic number and phagocytic index, and the value of NBT-test. The increase in phagocytic index in dogs of both groups recorded on the twelfth day of the experiment: the first group of animals to $46,83 \pm 0,91\%$, which is higher than before the experiment, by $13,7\%$ ($p < 0,05$). In dogs, the second group the rate was $43,33 \pm 1,05\%$, which is above the reference value of $4,4\%$, but $7,5\%$ less than in animals of the first group ($p < 0,05$). In the 24 th day of the experiment phagocytic index in dogs that were subject to hyaluronic acid and tryfuzol remained high ($46,5 \pm 0,85\%$), which exceeded the rate of the second experimental group ($41,83 \pm 1,05\%$) on $11,2\%$ ($p < 0,01$).

Test of nitroblue tetrazolium (NBT-test) reflects the level of activation kysnevozalezhnyh bactericidal mechanisms of phagocytic cells. Probable growth rates NBT test in animals recorded

on the twelfth day of wound healing. The animals of the first group it was $1,74 \pm 0,11$, which is twice higher than before the study ($p < 0.001$). In the second group of dogs, the figure also during this period increased to $1,46 \pm 0,07$, which is higher than the initial value of 1.8 times ($p < 0,05$). Follow-established decline in NBT-test in both groups of animals.

Increased NBT test in the early stages of wound healing in both groups indicate the change of functional metabolic activity of leukocytes. Reduced activity levels NBT test were recorded during regeneration and wound healing, ie on 24 th day of research.

Thus, the immunological parameters of both experimental groups on the sixth day study did not significantly differ. All animals were recorded likely reduce CD4 + T cells as a consequence - IRI also decreased. On the 12th day studies recorded significant differences between the control and experimental performance groups. The animals of the first group, which used to treat MIC-108 and CC, absolute lymphocytes (including T-and B-lymphocytes) was significantly higher than in animals of the second group. Among the regulatory subpopulations significantly increased the proportion of CD4 + T cells, thereby increased rate of immunoregulatory index. In contrast, the animals of the second group IRI was significantly lower during this period.

During healing (24 th c Research) recorded content difference CD8 + lymphocytes: in other animals (control) group, the figure was significantly higher than in dogs research, as a consequence - the immunoregulatory index was lower. Lower values of IRI in control animals compared with research may indicate chronic inflammation.

Conclusions:

1. Topical application of drugs and tryfuzolu hyaluronic acid in treatment of purulent wounds in dogs promotes healing by accelerating reparative processes.
2. Use tryfuzol and hyaluronic acid helps to restore the performance of specific and nonspecific resistance of the organism in a phase of regeneration and proliferation (12-day): elevated levels of T cells ($1816,5 \pm 116,04$ cells / ml), B-lymphocytes ($1148,5 \pm 84,12$ cells / ml), phagocytic index ($46,83 \pm 0,91\%$), phagocytic number ($5,5 \pm 0,22$) and NBT-test ($1,74 \pm 0,11$).

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