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Application of Complex Medication Based on Shungit and Laminaria in Veterinary Surgery

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Abstract

Despite the large amount of experimental and clinical research and the large number of improved methods and tools for the prevention and treatment of inflammatory diseases in animals, the question to find ways and means of therapy, which would satisfy such requirements as shortening the recovery period, reducing the time and cost of treatment, simplicity and security of medical procedures remains open and unresolved. This article presents the results of experimental studies on the application of ointment on the basis of shungite and kelp in comparison with traditional methods. It is shown that the ointment has a positive effect on the rate of regeneration of injured tissue and epithelialization of the wound defect, reducing the healing time of wounds in cattle and rabbits by five days on average compared to the control group. The use of experimental ointment helps to reduce bacterial contamination of wounds in rabbits. The number of microbial cells on the fifth day of treatment with the experimental medication was 400-500 microbes per 1 ml of outwash from the wound surface, while in the control group this index was at the level of 700-900. Thus, the experimental drug was found to have had a positive impact and can be recommended for use.

Keywords

Wound; Cattle; Rabbits; Treatment; Shungite; Kelp; Ointment

Introduction

Injuries of animals are widespread in animal husbandry, and high morbidity and mortality of farm animals hinder the growth of livestock production. Most injuries in animals are caused by a variety of mechanical effects; they average up to 70-80% of all surgical diseases. The most common mechanical injuries include bruises, wounds, burns, dislocations of joints, torn ligaments and tendons, broken bones and fractures and others. They are often complicated by a purulent inflammation and make up a significant percentage of surgical diseases. In view of the above it can be concluded that the search for effective, technically simple ways of complex treatment of animals is one of the most urgent problems of veterinary surgery. It is also necessary for such kind of medication to affect local inflammatory process and immune system simultaneously.

Wound healing is a well-coordinated biological process, which includes an integrated interaction of various growth factors, cytokines, enzymes and keratinocytes, fibroblasts, endothelial cells and others [1-4]. Such understanding of physiological processes has significantly increased during the last decades, mainly due to animals modeling [5-8].

Selecting an animal model is largely dependent on the purpose of study. Small animals as mice, rats and rabbits are suitable in studies that require large numbers of animals due to their availability, low cost and ease of handling. There are many anatomical and physiological differences between the human and, for example, mouse skin, such as wool, covering the mouse skin, lack of sweat glands, its thickness etc. [9-12]. A large number of experiments are carried out on pigs, because pig skin has a lot in common with the human one: a thick cuticle, abundant subcutaneous adipose tissue with collagen, similar hair follicles etc. [13-16]. Nontraditional methods and ways of wounds treatment are being used more and more often [17-20].

A review of the scientific literature shows that, despite the large amount of experimental and clinical research and development of

improved methods and tools for the prevention and treatment of inflammatory diseases in animals, the question to find ways and means of therapy remains open and unresolved. These methods should satisfy such requirements as recovery process shortening, reducing the time and cost of treatment, simplicity and security appliances of medical procedures.

Materials and Methods

Experimental studies on cattle were carried out on the basis of livestock farm in Kostanai oblast (Kazakhstan); experiments on rabbits were made in the veterinary clinic of Latvian State University (Jelgava, Latvia).

A comparative study of the effect of an experimental ointment on skin and muscle injuries and the morphological and biochemical indices of the blood of calves aged 6-12 months whose live weight was 150-200 kg was carried out.

Planar skin-muscle injury was applied to experimental animals with the use of the standard technique. Coat was cut out and shaved, surgical site was treated with 70% ethyl alcohol and 5% alcoholic solution of iodine. To apply equally sized wounds, a stencil made of a plastic plate was used. The stencil covered the surgical site and contours were applied along its edges with a pen. The operation was performed under local infiltration anesthesia. Place for planned defect was anesthetized with 0.5% solution of novocaine. Wounds were effected 10 min after

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anesthetic injection; effectiveness of anesthesia was pretested with injection needle prick. A scalpel and tweezers were used to remove cutaneous flaps with an additional cut of muscle of specified size. Hemostasis was performed with tight tamponade.

After stopping the bleeding, the wounds were infected. Gauze soaked in 30% slurry of faeces of cattle was put into the injury. Wounds healed by secondary intention, without being brought together with overlapping seams.

Animals selected for the experiment were divided into two groups of five animals each:

Group 1 (control). Festering wounds of these animals were treated with a solution of Furacilinum 1:5,000 in the first phase of healing process.

Group 2 (experimental). Festering wounds in the group of calves were treated with experimental ointment in the phase of hydration.

Planimetric studies of the wound surface were conducted on the first, third, seventh, eleventh, fifteenth and twentieth days. Transparent soft cellophane tape was applied to the wound. On the outside of the film, contours of the wound surface were circled with a soft-tip pen. Then the image was placed on graph paper and the number of square millimeters inside the circuit was counted. This figure was the area of the wound. Then daily decrease of injuries was determined through the formula:

$$\frac{S - S_n \times 100}{S \times t},$$

where: S is an area of the wound in the previous measurement; S_n is a wound area for a given dimension; t is the number of days between measurements.

Clinical examination of the calves and morphological parameters of blood were performed with conventional veterinary medicine schemes.

Study of size, length and depth of the wounds was performed as follows. There were inspected wounds, their edges (even, uneven, dropsical), it was noted the presence of scabs on the wounds, their color, how easy they are separated. It was determined the presence of epithelial rim, its color and size in millimeters, the appearance of granulation tissue in wounds, its character (pink, rough, pale pink, fine, bright red, coarse). Inspection of the walls and bottom of the wounds was made visually. The presence of swelling in the circle of wound, its size and consistency were noted.

Treatment was carried out daily after wounding. Wounds were mechanically cleaned with sterile cotton gauze moistened with sterile saline. The treatment was completed after full wound healing.

Rabbits were divided on the basis of paired analogs into two groups of six animals each. Planar skin-muscle injury with an area of 400 mm was applied to the animals in the withers.

After having stopped the bleeding, the wounds were infected with a mixture of soil and manure.

On the second day after the operation, a vivid picture of acute purulent inflammation was observed. The skin around the wound was characterized by hyperemia and edema; local temperature increased. The wound cavity was filled with exudate of pale yellow.

Treatment was carried on the second day after surgery.

Wounds of animals of the experimental group were treated with experimental ointment composed of vaseline (two portions), shungit (one portion), an extract of *Laminaria saccharina* with 50% ethanol (one portion).

Treatment of wounds of the rabbits in the control group was performed with ointment LMP (production by Riga, Latvia). The ointment, a gel-like opaque yellow paste, comprises surfactants: lidocaine hydrochloride, methyluracil, adjuvants: hinifuril, lecithin, ethyl alcohol 96%, carbomer, triethanolamine (trolamine), methyl parahydroxybenzoate, purified water. Lidocaine hydrochloride is a local anesthetic that relieves pain. Methyluracil is acting externally and promotes epithelialization and granulation of tissues, thus accelerating wound healing.

Medicines were applied to the wound with a gauze. Bandages were strengthened with the help of plaster. Inspection of wounds and change of bandages were performed daily until complete healing. The main methods of studies in rabbits were: measurement of the area of the wound surface, cytology of smear prints, bacteriological examination. Planimetric measurement was performed on the fourth, eighth and twelfth days after beginning treatment.

Cytological analysis of smearprints. Smears were prepared with gentle application of the sterile slide twice to the same study area of wounds (if the wound surface is abundantly covered with purulent exudate, it is removed with a sterile cotton gauze pad moistened with physiological sodium chloride solution). Prepared prints were dried in the air, then fixed in 96% ethanol (10 min) and stained according to Romanovsky-Gimza. Thereafter, the paint was removed by a gentle stream of water, the preparations were dried with filter paper and examined with a microscope "Biolam"; microscope magnification in each case was 400 \times . The selection of material was carried out on the third, fifth, seventh and tenth days from the beginning of treatment.

For bacteriological tests, material taken with sterile gauze from the wound surface was placed in a test tube with 2 ml of saline. The proportion of solution was 1:10 and 1:100. For this 1 ml of ablation was transferred to a flushing tube with 9 ml of saline; solution in proportion 1:10 was prepared. To determine the total number of bacteria in the solution 1:100 there were made probes in volume of 1 ml per three petri dishes by simultaneous fills.

With the use of the starting material, a probe was made on nutrient agar, nutrient broth, yolk and salt agar, 5% blood agar containing 5% of defibrinated sheep blood, Kitt-Tarocito isolate anaerobic microorganisms. Probes were made into two tubes: one was heated to 80 $^{\circ}$ C during 20 min, the other remained intact. All probes (dish, tubes) were placed in an incubator at a temperature of 37 $^{\circ}$ C for 24 h. To determine the type of bacteria, a microscopy study of grown colonies was performed, then they were painted by following the Gram method. The sensitivity to antibiotics of the gained cultures was determined by disk method. Taking the material was performed for the first time when the signs of purulent inflammation appeared, and then on the third, fifth, seventh and tenth days after the beginning of treatment. The digital material was statistically processed with a computer program "Statistika 6."

Results and Discussion

Comparative evaluation of different methods of treatment of calves with experimentally infected skin and muscle injuries

In the period from November to December 2013 at the cattle farm LLP "Timofeevka-Agro" conducted an experiment on the comparative

evaluation of therapeutic efficacy of the experimental ointment for treating inflamed musculocutaneous wounds in calves.

Wound healing began in one day after wounding. In the control group spraying of wounds with Furacilinum solution (1:5,000) was used; in the test group experimental ointment was used. On the first day the clinical conditions of wounds were of the same type in all groups: purulent exudate mixed with blood was observed; there was the presence of necrotic masses on the walls of the wounds, swelling and flushing of wounds, raising the local temperature, soreness.

In the control group swelling around the wound began to subside significantly on the seventh to eleventh days, and in the experimental group, on the fifth day. Granulation of wounds was observed in the test group on the fifth day, in the control group, on the seventh. Epithelialization of wounds in the control group began on the eleventh day, while in the experimental group, on the ninth day. Reduction of exudation was observed on the eleventh day in the control group, on the eighth day, in the experimental group. Exudate in the control group was gray-pink or gray-white, of mucous consistency, with unpleasant odor. In the experimental group exudate was red and white or yellow-green of creamy consistency with specific odor. On the fifteenth day in the experimental group the development of epithelial rim, a decrease in the area of the wound surface, exudation reducing were noted; in the control group exudation was observed, epithelialization was weakly expressed.

Wound healing in the control group was within 26 days on average; in the test one it took 21 days.

Changing wounds area in the control group occurred abruptly. On the first day the wounds area was 1012.2 mm², on the third day there was a significant increase in the area up to 1230.8 mm², on the seventh day wounds area reduced in size to 814.0 mm² and on the eleventh day the area was 728.0 mm² and on the twentieth day the wounds area reduced by 73.3%. In the experimental group of animals, wounds area changed gradually. Within 24 h after the operation the area of the wounds was 966.4 mm², on the third day there was a gradual decrease in injuries to 808.2 mm², the next day this trend continued: 527.6 mm², 468.0 mm² correspondingly on the seventh and eleventh days. In subsequent period, in particular on the twentieth day, there was a significant reduction in the wounds surface area by 92.9%.

Analyzing the data on changes in the wounds area, it can be concluded that the healing process is accelerated significantly faster in the experimental group.

Percentage of wound area reduction in the control group dropped on the third day to 10.8%, then increased on the seventh day to 12.7% and on the eleventh day the percentage of wound surface area reduction reached 2.9%. At the fifteenth and twentieth days the area of wounds tended to decrease daily and it was 7.3 and 8.6%, correspondingly. In the experimental group it rose to 8.2% on the third day, and on the seventh day it rose to 8.7%, then index followed by a sharp decrease on the eleventh day and it was already 3.2%. On the fifteenth and the twentieth days, the percentage of daily wound area reduction was 15.5 and 12.4%.

As a result, the percentage of area reduction of experimentally infected wounds in calves was higher in the experimental group, where the experimental ointment was used.

The clinical condition is essential for an objective assessment of the impact of wound healing in the overall health of animals.

The body temperature of the animals during the experiment ranged within the physiological norm. The maximum temperature rise was noted in the animals in the control group on the seventh day, which amounted to 3.9% comparing with the original data. In the test group temperature rose by 0.76°C on the seventh day. During the following period of examination, the body temperature decreased and ranged within the indices of the original data.

Respiratory rate in animals increased to maximum on the first day after having wounded in the control group by 40.1% comparing with baseline, but in the test group respiration rate reached the maximum on the eleventh day and was 22.0 ± 0.89 ($p < 0.05$) and 23.2 ± 2.33 breaths per minute. Then respiratory rate decreased reaching a minimum value on the twentieth day in all groups.

Pulse rate in groups before injury ranged 79.6-87.2 beats/min. Then heart rate increased reaching the maximum data on the first fifteen days by 24.6% in the control group, by 22.9% in the test group on the third day. Further frequency variations were observed in heart rate within the background values.

Comparative characteristics of the infected wounds treatment in rabbits

For studies rabbits were divided into two groups of six animals each. Planar skin-muscle injury of 400 mm² area was applied in rabbits' withers. After stopping bleeding, wounds were infected with a mixture of soil and manure. The wounds began to be treated on the second day after surgery. In animals of the experimental group they were treated with the experimental ointment on the basis of shungit and kelp. The rabbits in the control group were cured with ointment for wounds treatment LMP.

While examination before treatment (on the second day after the injury application), in the control and experimental groups such signs of inflammation as redness and skin inflammation, swelling around the wound, moderately hot skin around the wound while providing palpation, pale yellow exudate in the wound cavity were determined visually and histologically.

Necrotic tissue could be seen on the histo-preparations in the damaged area, infiltration of stab and segmented leukocytes, lymphocytes were pronounced, plasma cells were found, in stromal components there were fibroblasts, stromal edema.

In wounds smears in this period among structureless mass, fragments of cells, deformed red blood cells there were lymphocytes, segmented and stab neutrophils, part of which was in a state of decay.

On the third day of treatment, there was a significant reduction in skin redness and swelling around the wound inflammation in the treated group; similar changes were observed in the control group on the fourth day. There was an active wound cleansing from necrotic masses.

Cleansing wounds from necrotic tissue in the experimental group was faster than in the control one. The appearance of granulation tissue in the wounds of the experimental group was observed on the third to fourth days after the start of treatment, and in the control one, on the fourth to fifth days. While treating with the use of the experimental ointment granulation appeared earlier, it had a red, coarse appearance. In the control group, granulation was pale, fine-grained, sometimes bleeding.

On the fifth day of treatment in the treated group, the number of neutrophils decreased and the number of macrophages increased.

Similar changes in the wounds of the control group were observed only on the seventh day of treatment.

On the tenth day of treatment with the use of the ointment on a collagen-based cytological pattern was characterized by a predominance of fibroblast cell elements, which indicated high activity of reparative processes.

In the smears of control group fibroblasts and macrophages prevailed.

Histology on the fifth day of wounds treatment with experimental ointment was characterized by the development of high-quality granulation tissue, increasing the number of mature fibroblasts, accumulation of histiocytes. In the wounds of control animals, leukocyte infiltration, plasma cells, proliferation of fibroblasts and endothelial components, stromal edema were more pronounced. On the thirteenth to fourteenth days of treatment, the wounds in rabbits of the experimental group were almost completely covered with epithelium, and complete healing was observed on the fourteenth to fifteenth h.

In the experimental group on the eighth to tenth days of purulent wounds treatment, more pronounced positive dynamics was observed. The process of necrotic mass casting off from the surface of the wound could be clearly seen. Basically it was filled with tissue rich in blood vessels. Growth of epithelium was more rapid than in wounds in the control group.

In the control group on the thirteenth to fourteenth days, the wounds were covered with dense, dry crust that separated on the nineteenth to twentieth days, forming a scar of pale red.

Based on the clinical and morphological characteristics of the wound healing process, it can be noted that the most complete regeneration of skin and muscle was observed within the treatment of wounds with ointment on the basis of collagen.

Speed of wound area reduction determines the length of the third phase of regeneration and is an important clinical indicator of wound healing speed.

In two days after wounds application on the skin and muscle, the average area of which was 401.3 ± 4.7 mm in the treated group and 396.3 ± 5.9 mm in the control group; signs of inflammation were observed. The skin around the wound was characterized with hyperemia and edema; local temperature increased.

Statistical processing revealed that the difference in the initial wound area in experimental and control groups are negligible, so the wounds can be regarded as substantially identical.

During the first day of treatment, the average wound area in the control and experimental groups was nearly equal to the original data.

Active epithelialization of the wound edges was observed on the eight day after beginning treatment. By this period wounds area in rabbits of the experimental group was reduced by an average of $58.6 \pm 0.9\%$ and was 166.3 ± 3.8 mm², and the wound area decreased by an average of $13.7 \pm 0.2\%$ per day. In this period in the animals of the control group, the average area of the wounds was equal to 248.7 ± 6.9 mm² (declining by $37.3 \pm 0.69\%$) and it decreased by $8.4 \pm 0.17\%$ per day. On the twelfth day of treatment, the average area of the wounds in the experimental animals was 51.0 ± 2.0 mm (reduced by $87.3 \pm 0.8\%$); complete healing was observed on fourteenth to fifteenth days.

Complete healing of wounds in the control group was observed on the twentieth day. These data show that the treatment with experimental ointment provides wound area reduction as well as the wound area reduction percentage relative to the initial in absolute terms occurs significantly faster than within the treatment in the control group.

Evaluation of the results of bacteriological examination of wound exudate

The study of the outwash of the wound surface of rabbits revealed the presence of *Staphylococcus aureus*, *Staphylococcus epidermidis*, soil bacillus *Proteus* and other microbes.

In addition to typing of isolated culture, the total number of bacterial colonies was counted and the sensitivity of cultures to antibiotics was determined.

The level of bacterial contamination of wounds before treatment in both groups was 1,000-1,500 microbial cells in 1 ml of outwash. While the wounds treatment in the control group during the first three days the number of microorganisms had not changed. On the fifth day of treatment, this figure dropped to 700-900 microbial bodies.

Within the treatment of wounds with experimental ointment reduction of bacterial contamination occurred much faster.

On the third day of treatment, the number of microbial cells in 1 ml of outwash was within the number of 700-1,000; on the fifth day it dropped to 400-500 microorganisms and remained at this level in subsequent studies.

The results of determining the sensitivity of isolates to antibiotics showed that these microorganisms were sensitive to cephalosporin antibiotics (Cefazolinum, ceftriaxone), fluoroquinolones (ciprofloxacin, ofloxacin), but resistant to sulfanilamides (Sulfazinum).

Change of some physiological parameters of rabbits

In order to assess the general condition of the body pulse, number of respiratory movements and body temperature were measured.

These figures were recorded throughout the treatment period, twice in the morning and evening; daily average figures were counted.

Throughout the time of research breathing acceleration of rabbits was recorded. In our opinion it is connected with the development of traumatic stress in the period of a scientific experiment. Maintenance of homeostasis in the body of animals is carried out through the processes of heat production and heat loss. The skin plays an important role in thermoregulation, since about 60% of the total heat losses through evaporation occur due to skin. This is facilitated by the sudoriferous glands. In rabbits, due to the weak development of the sudoriferous glands, water evaporates mainly through the respiratory tract.

The analysis showed that there was a slight increase (not going beyond physiological limits) of the pulse rate on the third day after the injury application. Then this figure gradually began to decline.

In rabbits changes in body temperature are similar to the pulse rate fluctuations. In general in groups there was an increase (within limits) of the body temperature in the first two days after surgery, and then it declined gradually.

Thus, application of the experimental ointment on the basis of shungite and kelp had an antiinflammatory effect manifested with a terms reduction of body reaction to injury.

Conclusion

The main role in the occurrence of purulent inflammation of skin and muscle injuries regardless of the healing phase and season is played by *Streptococcus pyogenes*, *Staphylococcus saprophyticus*, *Escherichia coli* and bacteria of the genus, *Proteus*. Most of isolated micro flora strains showed high sensitivity to Cefotaxime (87.2%) and kanamycin (79.5%).

Ointment based on shungite and kelp has a positive effect on the rate of regeneration of injured tissue and epithelialization of the wound defect; the time of wounds healing in cattle and rabbits reduces on average by five days compared with the control group.

Application of ointments based on shungite and kelp helps to reduce bacterial contamination of wounds in rabbits. Number of microbial cells on the fifth day of treatment with the collagen preparation was 400-500 microbes per 1 ml of the wound surface outwash, while in the control group this index was at the level of 700-900.

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