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ВЕТЕРИНАРЕН

Ветеринарна медицина

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METHODS FOR ACCUMULATING MEDICINAL PREPARATIONS FOR VETERINARY PURPOSES.

Currently, in connection with the development of new technological approaches to the creation of basic drugs for the treatment of joint diseases and evaluation of their mechanism of action on articular cartilage, the terms “chondromodulating drugs” or “structural−modifying drugs” are used. This implies that along with the chondroprotective effect they can have effect on the metabolism of articular cartilage. [2]

Traumatic injuries, microtraumas, inappropriate intensity of mechanical load are manifested in all subsystems of the articular apparatus: ligaments, capsule, articular cartilage and can cause microtraumatization with the subsequent development of arthritic disorders [2,5].

Joint injury always leads to damage to the articular cartilage to some extent. Dystrophic altered or damaged areas of the articular surface of the bone gradually lose their luster, thin out, and become covered with star-shaped cracks. This pathological condition of cartilage tissue is called traumatic chondromalacia [3].

Various ointments and gels can be recommended for long-term effects directly on the joint area.

Currently, preparations based on low-molecular amino sugars (glucosamine) and high-molecular polysaccharides (chondroitin sulfate, hyaluronic acid), as well as combined preparations based on glucosamine and chondroitin sulfate, sometimes with additional additives, are widely used.

The drugs used for the treatment of osteoarthritis have proven clinical effectiveness and proven beneficial effect on cartilage.

Dosage formulations containing glucosamine and chondroitin stimulate the synthesis of articular proteoglycans. In addition, glucosamine shows anti-inflammatory properties, slows down the degradation of articular cartilage mainly due to its metabolic activity, the ability to suppress the activity of interleukin (IL)-1, lysosomal enzymes, collagenase and phospholipase A2. The effect of treatment with glucosamine sulfate is visible in 2 weeks from the start of treatment. The question remains, how and in what order to use chondroprotective drugs to normalize the work of articular structures. [2,4]

Material and methods of research

The experimental research material consisted of 25 sexually mature mongrel eight-month-old male rats. Before starting the experiment, the animals were divided into 5 groups of five in each group. The left knee joint was selected for experimental observation.

The rats of the first group were intramuscularly injected with the drug Sinarta. The calculation of the amount of the administered substance was carried out taking into account the average weight of animals. The average weight of animals was 100 g. The medication was administered every alternate day, as recommended by the instructions for use of this medication. The entire course consisted of 10 injections.

For the rats of this group, Chondroxide gel was additionally applied on the knee joint daily for 20 days.

The rats of the second group received intramuscular injections of Sinarta and electrophoresis of Chondroxide gel on the knee joint every alternate day. Electrophoresis was performed as follows: Chondroxide gel was applied to the shaved knee joint, the active electrodes were attached parallel to each other (this condition was mandatory, so that the active substance penetrated the joint structures as much as possible). The passive electrode was attached to the shaved part of the spino-caudal area. Electrophoresis was performed for 7 minutes, with a current of 0.5 A, in total 10

sessions was conducted.

The rats of the third group received only intramuscular injections of Sinarta thatwasadministered according to the same scheme used for the first and second groups.

The rats of the fourth group received onlyChondroxide gel, whichwas applied twice a day for 20 days, as recommended by the instructions for use of this medication.

The fifth group of animals was a control group, so it was kept in normal standard conditions.

After 21 days, the rats were withdrawn from the experiment. The animals were culled through decapitation under ether anesthesia in accordance with “The guidelines for withdrawing of animals from the experiment” [1].

After withdrawing the animals from the experiment, the knee joint, on which medical measures were performed, was isolatedand in groups 3 and 5, the left knee joint was isolated, as described above.

To prepare knee joint homogenates from samples gathered from experimental groups, the joints were freed from the skin and ground in a mortar. The mortar and pestle were washed and wiped dry after each crushed joint. The crushed joints were placed in test tubes according to the groups, 1 ml of saline solution was added to each tube, mixed, and allowed to settle in the refrigerator at a temperature of +3 C for a day. After a day, centrifugation was performed at 3000 rpm for 15 minutes.

A dye was prepared that consisted of 55 mmol of formic acid in 200 ml of saline solution and 2.1 mg of methylene blue (0.5 ml of formic acid 85 % and diluted with saline solution up to 200 ml).

Staining and spectrophotometry with a wavelength of 520 nm were performed to determine the amount of glycosaminoglycans in the prepared solutions.

To do this, 2.5 ml of the dye (the solution was stable for 3 minutes) was added to 0.1 ml of the supernatant fluid from the centrifugated homogenate of the knee joint. The spectrophotometry ofthis material was performed. The result was obtained in digital values of optical density; then the obtained values were compared between the experimental groups and the control group.

To determine the method of administration and the onset of the therapeutic effect associated with the cumulative effect of sulfated glucosaminoglycans on the joint structures, chondroprotective drugs were used: Sinarta and Chondroxide gel.

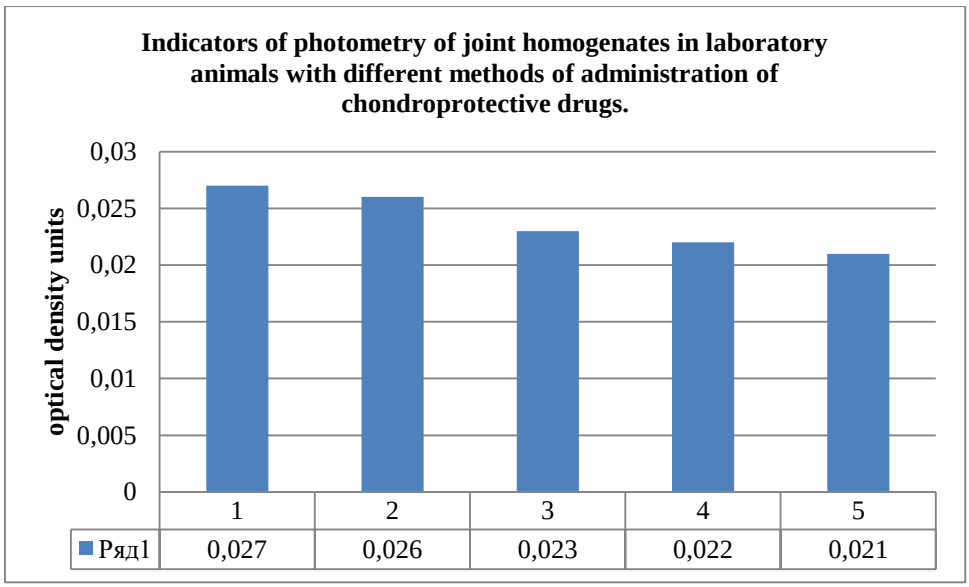
Sinarta — anti-inflammatory agent, eliminates endogenous glucosamine deficiency, stimulates the synthesis of proteoglycans and hyaluronic acid in the synovial fluid; increases the permeability of the joint capsule, restores the enzymatic processes in the cells of the synovial membrane and articular cartilage. It encourages thiopexy in the process of synthesis of chondroitinseric acid, promotes calcification of bone tissue, inhibits the development of degenerative processes in the joints in cases of diseases, restores their function, reduces the severity of arthralgia.

Chondroxide gel is a medication for external use that improves the regeneration of cartilage tissue and has the anti-inflammatory effect. It is stimulator of tissue regeneration. Chondroxide normalizes metabolism in hyaline tissue, stimulates regenerative (restorative) processes in articular cartilage, has analgesic and anti-inflammatory effects, slows down the progression of osteoarthritis and osteochondrosis. The medication contains a natural component of chondroitin sulfate, obtained from bovine cartilage tissue. It is a high-molecular mucopolysaccharide that slows down the resorption of bone tissue and reduces calcium loss, improves the phosphorus-calcium exchange in cartilage tissue, accelerates its repair processes, and slows down the process of degeneration of cartilage tissue. It prevents the collapse of connective tissue. It inhibits enzymes that cause damage to cartilage tissue, stimulates the synthesis of glycosaminglicans, promotes the regeneration of the articular bag and cartilage surfaces of joints and increases the production of intra-articular fluid.

Results and discussion

Based on the analysis of the results of the research, the maximum optical density of the studied supraventricular fluid of knee joint homogenates was obtained in the first and second groups: 0.027±0.0008 and 0.026±0.004, respectively. These groups received parenteral administration of Sinarta with additional application of Chondroxide gel in the first group and electrophoresis with Chondroxide gel in the second group.

The third and fourth groups, which received only intramuscular administration of Sinarta and only application of Chondroxide gel on the knee joint, respectively, showed increase in optical density of the supernatant liquid of homogenates of the joints in comparison to the control group and no significant increase in comparison to the first and second groups. The digital values were distributed as follows: the third group 0.023±0.0009, the fourth group 0.022±0.0004, the fifth group-the control group 0.021±0.001 (optical density units).



Ряд –Row

The accuracy of differences in the indicators P≤0.05 between the first two groups was obtained through comparison with the third, fourth and fifth groups, respectively. No accuracy of differences in the indicatorsP≥0.05 wasdeterminedbetween the first and second groups, as well as between the third and fourth groups. The accuracy of differences in indicators between the first two groups with indicators of the third and fourth groups indicates the effectiveness of the combined method of usage of chondroprotective drugs.

The above presented research took into account the causes of joint diseases and pathogenesis links that need to be treated by the combined method of administration of chondroprotectors.

Arthrosis. It develops in cases, when the cartilage and subchondral bone are not able to adequately resist mechanical stress, which is associated with a limitation of the reparative capabilities of these tissues. The main foothold for the development of pathological changes is hyaline cartilage, in which suffers not only from a decrease in the number of chondrocytes, but also from a decrease in their metabolic activity. This leads to a decrease in the synthesis of collagen in the cartilage matrix and sulfated proteoglycans – chondroitin sulfate, keratan sulfate, proteoglycan-hyaluronic aggregates and hyaluronic acid. The most important component of these changes is a deficiency in the synthesis of proteoglycans, the main structural component of the cartilage matrix. Arthrosisnot only reduces the quantitative synthesis of proteoglycans, but also changes their qualitative composition, namely, the production of full-fledged proteoglycans with high molecular weight. [6,]

Arthritis. It is a heterogeneous group of diseases with inflammatory and degenerative changes in the entire tissue complex of the joint: cartilage, subchondral bone, synovial membrane, ligaments, capsules, periarticular tendons and muscles, but the most serious changes occur in the cartilage tissue. The cartilage tissue that is experiencing significant mechanical loads is forced to constantly self-renew, this process is provided by the chondrocyte system. Their function is to reconstruct the connective tissue matrix, the main components of which are collagen and proteoglycans. In arthritis, chondrocyte renewal is disrupted and, as a result, destructive processes in the matrix prevail over restorative ones. [6]

Based on our research, it has been shown that the maximum cumulative and therapeutic effect of medication occurs with a combined method of medication administration, namely: parenteral route and local action in the joint area during gel application or electrophoresis.

Conclusions

1. The most effective way to accumulate glucosamines in the structures of the joint is a combination of intramuscular administration of the medication and local administration throughapplication or electrophoresis.

2. Digital values of the optical density of joint homogenates indicate the effectiveness of the cumulative method of administration of glucosamines and chondroitin sulfates into the joint structures.

3. Significant differences were obtained when using different methods of administration of medicinal substances of chondroprotective action on the structures of the joint.

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