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NON-INVASIVE METHODS FOR INTRODUCING CHONDROPROTECTIVE DRUGS INTO KNEE JOINT OF RATS

Abstract.

A comparative analysis of different ways of introducing chondroprotectors into the knee joint in laboratory animals was performed.

25 sexually mature, outbred, eight-month old male rats served as a material for the experimental study performed. Before the experiment began, the animals were divided into 5 groups; each group consisted of five rats. The left knee joint was selected for the experimental observation.

Based on the analysis of the results of the study, the maximum optical density of the knee joint homogenates was obtained in the first and second groups. In these groups, parenteral-intramuscular administration of Sinarta was performed, as well as additional rubbing of Chondroxide gel in the first group and electrophoresis with Chondroxide gel in the second group.

Reliable differences in the effects on joint structures were obtained when using different methods of administering chondroprotective medicinal substances. The most effective way of accumulation of glucosamine within the joints is a combination of intramuscular administration of the drug and topical administration by rubbing or electrophoresis. The numeral values of the optical density of joints' homogenates indicate the effectiveness of the cumulative method of administering glucosamines and chondroitin sulfates into the structures of joints.

Key words: Laboratory animals (rats), chondroprotective drugs, route of administration of drugs.

Traumatic injuries, microtraumas, as well as discordance in the mechanical loads manifest themselves in all subsystems of the articular apparatus, including ligaments, capsule, articular cartilage, and can cause micro-traumatization with the subsequent development of arthrosis-type disorders [2,5].

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Trauma to joints always in varying degrees leads to damage of the articular cartilage. Dystrophic, altered or crumpled areas of the articular surface of the bone gradually lose their luster, get thin and become covered with stellate-shaped cracks. This pathological condition of the cartilage tissue is called traumatic chondromalacia [3].

Currently, in connection with the development of new technological approaches to the creation of basic drugs for treatment of diseases of the joints and assessing their mechanism of influence upon articular cartilage, the terms “chondromodulating drugs” or “structure-modifying drugs” are used. This implies that, along with chondroprotective effect, they can influence the metabolism of articular cartilage. [2]

In cases when long-term direct exposure of the joint area is required, various ointments and gels can be recommended.

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

Drugs used to treat osteoarthrosis have proven clinical efficacy, and a proven beneficial effect on cartilage.

Dosage forms containing glycosamines and chondroitins within their composition stimulate the synthesis of articular proteoglycans. In addition to that, glucosamine exhibits anti-inflammatory properties, slows down the processes of degradation of articular cartilage mainly due to its metabolic activity, its ability to suppress the activity of interleukin (IL)-1, lysosomal enzymes, collagenase and phospholipase A2. The effect of treatment with glucosamine sulfate appears after 2 weeks from the start of treatment. It remains an open question how and in what sequence chondroprotective drugs should be applied in order to normalize the functioning of structures of the joints. [2,4]

Material and methods of research

25 sexually mature, outbred, eight-month old male rats were selected as a material for the experimental study to be performed. Before the experiment began,

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the animals were divided into 5 groups; each group consisted of five rats. The left knee joint was selected for the experimental observation.

In the first group, Sinarta was injected intramuscularly in rats. The calculation of the amount of substance to be injected was carried out taking into account the average weight of the animals. The average weight of the animals was 100 g. The drug was administered once in two days, as recommended by the instructions for use of this medicine. The entire course was 10 injections.

Additionally in this group, Chondroxide gel was rubbed into the knee joint on a daily basis during 20 days.

In the second group, the animals were given intramuscular injections of Sinarta once in two days and an electrophoresis of Chondroxide gel on the knee joint. Electrophoresis was carried out as follows: Chondroxide gel was applied to the shaven knee joint; the active electrodes were installed parallel to each other; this condition was obligatory, so that the active substance penetrated the structures of the joint as much as possible. The passive electrode was installed on the shaved part of the spin caudal section. Electrophoresis was carried out for 7 minutes, with a current intensity of 0.5 A.

In the third group, only intramuscular injections of Sinarta were performed according to the same scheme as in the first and second groups.

In the fourth group, only Chondroxide gel was rubbed in twice a day for 20 days, as recommended by the instructions for use of this medicine.

The fifth group of animals was a control group, kept in the usual standard conditions.

After 21 days, the rats were removed from the experiment. The slaughter of animals was carried out by decapitation under ether anesthesia in accordance with the “Methodical recommendations for the removal of animals from the experiment” [1].

After the animals were removed from the experiment, the knee joint was isolated, where therapeutic measures were taken, and in groups No. 3 and No. 5, the left knee joint was isolated, as specified above.

For the preparation of homogenates of the knee joint in the experimental groups, the joints were freed from the skin and pounded in a mortar. The mortar and

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pestle was washed and wiped dry after each crushed joint. The crushed joints were laid out in test tubes according to the groups, 1 ml of saline solution was added to each tube, mixed, and allowed to stand in a refrigerator at a temperature of +3 ° C for 24 hours. After one day, centrifugation was performed at 3000 rpm for 15 minutes.

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

In order to determine the amount of glycosaminoglycans in the prepared solutions, staining and spectrophotometry with a wavelength of 520 nm were performed.

For this purpose, 0.1 ml of the supernatant from the centrifuged knee joint homogenate was taken and 2.5 ml of dye was added (solution stable for 3 min) and then spectrophotometry was performed. The result was obtained in numeral values of optical density and later the obtained values were compared between the experimental groups and the control group.

In order to determine the method of administration and the onset of a therapeutic effect reached due to the cumulative effect of sulfated glycosaminoglycans on the structures of the joint, chondroprotective agents – Sinarta and Hondroxid gel – were used.

Sinarta is an anti-inflammatory agent that fills up the endogenous deficiency of glucosamine, stimulates the synthesis of proteoglycans and hyaluronic acid in the synovial fluid; increases the permeability of the articular capsule, restores enzymatic processes in the cells of the synovial membrane and articular cartilage. It also promotes sulfur fixation in the process of chondroitin sulfuric acid synthesis, facilitates bone tissue calcification, inhibits the development of degenerative processes in the joints during their diseases, restores their function, reduces arthralgia severity.

Chondroxide gel is a preparation for external use that causes anti-inflammatory effect and improves cartilage tissue regeneration. It is a stimulator of tissue regeneration. Hondroxide normalizes metabolism within hyaline tissue, stimulates regenerative (restorative) processes in articular cartilage, has an analgesic and anti-

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inflammatory effect, and slows the progression of osteoarthrosis and osteochondrosis. The drug contains a natural component of chondroitin sulfate, derived from the cartilaginous tissues of cattle. It is a high-molecular mucopolysaccharide, which slows down bone resorption and reduces calcium loss, improves phosphorus-calcium metabolism in cartilage, accelerates its repair processes, and slows down the process of cartilage tissue degeneration. It prevents the collapse of connective tissue. It also inhibits enzymes that cause damage to the cartilage tissue, stimulates the synthesis of glycosaminoglycans, facilitates the regeneration of the articular sac and the cartilage surfaces of the joints, increases the production of intraarticular fluid.

Results and their discussion

Based on the analysis of the results of the research, the maximum optical density of the examined knee-joint homogenates was obtained in the first and second groups: 0.027±0.0008 and 0.026±0.004, respectively. In these groups, parenteral administration of Sinarta was performed, as well as additional rubbing of the Chondroxide gel in the first group and electrophoresis with Chondroxide gel in the second group.

In the third and fourth groups, where only intramuscular administration of Sinarta was used and only Chondroxide gel was rubbed into the knee joint, an increase in the optical density of joint homogenates relative to the control group and a slight increase in relation to the first and second groups were obtained. The numerical values were distributed as follows: 0.023 ± 0.0009 in the third group, 0.022 ± 0.0004 in the fourth group, 0.021 ± 0.001 (units of optical density) in the fifth control group. Fig.

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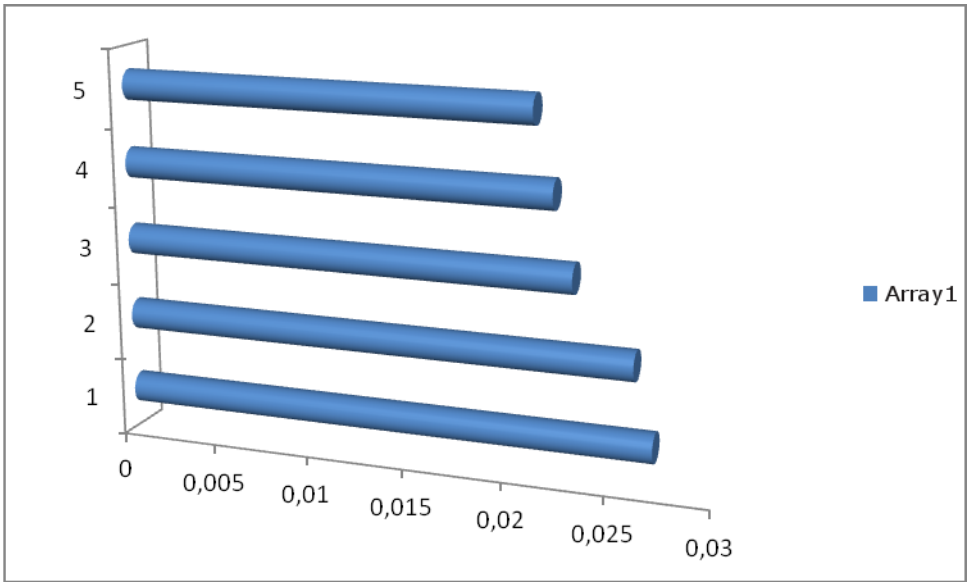

Fig. Rates of photometry of knee-joint homogenates in rats.

Reliability of differences P≤0.05 between the first two groups was obtained when compared with the third, fourth and fifth groups, respectively. No reliability of differences was established P≥0.05 between the first and second groups, as well as between the third and fourth groups. The reliability of differences between the indicators of first two groups and the indicators of the third and fourth groups proves the effectiveness of the combined method of applying chondroprotective drugs.

In the above research, the causes of the development of diseases of the joints and pathogenesis links, that need to be influenced by the combined method of introducing chondroprotectors, have been studies.

Based on our research, it was shown that the maximum cumulative and therapeutic effect of drugs is reached when using a combined method of administering drugs, namely: parenterally and by way of local exposure in the area of the joint during rubbing procedures or electrophoresis.

Findings

1. Significant differences were obtained when using different methods of introducing medicinal substances that cause chondroprotective effect on the structures of the joint.

2. The most effective way of accumulating glucosamines in the structures of the joints is a combination of intramuscular administration of the drug and local introduction by rubbing or electrophoresis.

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3. Numeral values of the optical density of joint homogenates indicate the effectiveness of the cumulative method of introducing glucosamines and chondroitin sulfates into the joint structures.

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