

## THE EFFECT OF GLUCOSAMINE HYDROCHLORIDE AND SODIUM DICLOFENAC WITH LASER RADIATION ON EXPERIMENTAL KNEE OSTEOARTHRITIS IN COMPLEX TREATMENT OF RATS

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The modern approach to the integrated therapy of knee osteoarthritis is grounded on the recommendations of EULAR [2], built on the principles of evidence-based medicine. All approaches to the treatment of patients with knee joints osteoarthritis were divided into 4 groups: pharmacological, non-pharmacological, intra-articular and surgical. The pharmacological agents most commonly used to treat osteoarthritis of the knee joints are nonsteroidal anti-inflammatory drugs. They have the ability to reduce pain and show a pronounced anti-inflammatory effect [3]. In clinical practice, more and more clinicians are using drugs based on glucosamine hydrochloride. Its bioavailability is about 90%. It has pronounced chondroprotective properties, moderate anti-inflammatory effect, and prevents the progression of degenerative processes in the joint [5]. In recent years, the attention of specialists is also attracted by the possibility of using laser therapy in the treatment of patients with knee joints osteoarthritis with severe articular syndrome. The use of laser radiation in the treatment of knee osteoarthritis provides significant anti-inflammatory and analgesic effects [4]. This is accompanied by changes in such biochemical markers of blood as glycoproteins and proteoglycans which reflect the state of joints connective tissue. The catabolism of these markers varies depending on the course of inflammatory and reparative processes in joints osteoarthritis. Thus, a rational combination of local effects and general therapeutic measures is required in the regenerative treatment of patients with osteoarthritis. In this case, the effectiveness of complex conservative treatment does not depend only on the correct choice of laser irradiation parameters, which are mainly aimed at reducing the intensity of inflammation and the elimination of pain syndrome [1].

**Aim** – experimental definition of the effectiveness of the proposed method of conservative treatment of knee joints osteoarthritis using sodium diclofenac, glucosamine hydrochloride and infrared laser radiation, on the basis of biochemical markers of connective tissue metabolism in rats.

**Materials and methods.** Experimental studies were performed on 40 white male rats aged 6 months with body weight from 250 to 300 g of the population of the experimental and biological clinic of Sytenko Institute of Spine and Joint Pathology. Animals were kept in accordance with sanitary norms on the standard diet. Experiments on rats were performed within regulations of the European Convention for the Protection of Vertebrate Animals used for experimental and other purposes. Euthanasia of animals was carried out by decapitation due to the need of blood selection for the research. Knee joints osteoarthritis was modeled by intramuscular administration of dexamethasone at a dose of 7 mg/kg, once a week during 3 weeks. The treatment started 3 days after the last injection of dexamethasone. Experimental group consisted of 5 rats whose biochemical blood tests results had been initially evaluated after 3 weeks of knee joints osteoarthritis. A control group had 5 intact rats. Group I (n = 10) comprised rats with simulated knee joints osteoarthritis without further treatment. They were observed on the 10<sup>th</sup> and 24<sup>th</sup> days. Group II animals (n = 10) were treated by administering diclofenac sodium (8 mg/kg) and glucosamine hydrochloride (50 mg/kg) orally. Drugs were injected per os with a probe. The volume of the solution was 0.5 ml/100 g of a rat weight. Group III animals (n = 10) received infrared laser irradiation at a dose of 0.3 J followed by oral administration of diclofenac sodium (8 mg/kg) and glucosamine hydrochloride (50 mg/kg).

Laser therapy was performed on the caudal surface of the right wool-free knee joint using the Mustang apparatus: wavelength 0.89  $\mu\text{m}$ , pulsed power 7-8 W, pulse frequency 3000 Hz, the session duration was 3 minutes 42 seconds. Laser sessions were performed daily during 10 days (10 sessions). To evaluate the treatment efficacy of the proposed schemes we carried out a biochemical study of rats' blood treated with different regimens on the 10<sup>th</sup> and 24<sup>th</sup> days of treatment. Glycoproteins, chondroitin sulfates, GAG fractions, alkaline phosphatase were identified in the blood. Statistical data analysis was carried out using software packages Microsoft Excel XP and Statsoft Statistica 10.0. Comparison of patient groups was performed according to the Student's parametric criterion and Wilcoxon's non-parametric criterion.

**Results and discussion.** On the 24<sup>th</sup> day of the recovery period, after the simulating knee joints osteoarthritis, we detected an increase in the content of the connective tissue metabolism markers (glycoproteins, chondroitin sulfates) and the change in the ratio of GAG fractions in blood serum of rats. It is known that virtually all GAG cartilage matrices, except for hyaluronan, are sulfated. They are chondroitin sulfate and keratan sulfate. The total concentration of GAG in the matrix of articular cartilage reaches 10 g/100 g of dry matter, which during the development of inflammatory-destructive processes in the cartilage of the knee joints can lead to increasing of their concentration in blood serum. The concentration of glycoproteins in blood serum of rats with experimental knee joints osteoarthritis increased by 37.0% compared to the indicator in the intact group of animals. In our opinion, this is due to the inflammatory process in the capsule of the affected joints. Cartilage tissue of the joints is rich in glycoproteins, which, in case of osteoarthritis, causes an increase in their expression by chondrocytes. These include fibronectin, tenascin-C, clusterin, proteoglycan-binding protein and other glycoproteins involved in cartilage catabolism in inflammatory-degradation processes in the joints during inflammation. The destruction of cartilaginous tissue was confirmed by increase in the total chondroitin sulfates concentration by 32.9%, as well as GAG fraction I increase by 32.2%, GAG fraction II (chondroitin-6 and chondroitin-4-sulfates) increase by 79.8%, which are the parts of the cartilage and bone tissue of the affected joints. The obtained data show that the above-mentioned biochemical parameters fully reflect the processes occurring in the tissues of the affected joint. This is the reason for using the above-mentioned biochemical markers for laboratory control of the effectiveness of treatment. In the dynamics of treatment after 10 days of therapy, the content of glycoproteins decreased by 11.5% in the group of rats treated without laser radiation and by 23.7% in the blood of rats treated with laser therapy compared to the group of animals without medical treatment. It was also found that the content of blood serum glycoproteins in rats treated with diclofenac, glucosamine hydrochloride in combination with laser therapy was lower by 13.8% compared with the group of animals treated with drugs only. This is evidently due to the use of low-intensity laser radiation, which positively affects the tissues of the affected joints in knee osteoarthritis, further reducing the intensity of inflammation in the synovial membranes, edema, and promoting the regeneration of articular cartilage. Reduced catabolism of proteoglycans of articular cartilage caused a positive dynamics of chondroitin sulfate content in rats' blood with experimental knee joints osteoarthritis. In the group of animals treated without laser therapy, the content of total chondroitin sulfates was reduced by only 8.5% compared with the group without treatment. In the third group of animals after additional application of laser therapy, the decrease in the content of total chondroitin sulfates was 21.6%, which confirms the higher efficiency treatment regimens using laser therapy.

Moreover, rats treated with diclofenac and glucosamine hydrochloride with using laser therapy showed positive dynamics in the content of GAG fractions in blood serum. Lower values of I and II GAG fractions (chondroitin-6 and chondroitin-4-sulfate) were noted in this group of rats on the 10<sup>th</sup> day immediately after treatment, compared to the group II animals treated without laser therapy. There was also a decrease in serum alkaline phosphatase activity in the group of rats treated

with low-intensity laser radiation. Perhaps it was due to bone isoenzymes, indicating a slowing down of destructive processes in the subchondral bone of the joints in osteoarthritis. The content of total calcium in all animal groups remained unchanged compared to intact animals. A study of connective tissue biochemical markers in blood serum of rats was carried out after 24 days of the medical treatment course to assess the long-term results of rats' treatment by different methods of therapy. In the blood serum of rats treated with a combination of laser therapy and medical preparations, there was a significant decrease in the content of glycoproteins (28.5%), chondroitin sulfates (26.1%), alkaline phosphatase activity (32.3%), and I and II GAG fractions (chondroitin-6 (28.7%) and chondroitin-4-sulfate (40.3%)) 24 days after the treatment. We also noted that group III animals had approximately the same values of biochemical markers as intact animals, which is due to the greater effectiveness of the therapeutic technique with the use of the above-mentioned complex in comparison with using only drugs. In our opinion, the reason for such positive changes is precisely the action of laser beams, which contribute to the increase of local reactivity around the articular tissues, contribute to the reduction of the inflammatory process in the synovial membranes, and also create favorable conditions for the regeneration of articular cartilage. Thus, during the analysis of the results of biochemical studies of connective tissue markers in the blood of experimental animals, it was found out that the decrease in the activity of the inflammatory processes and the destruction of the cartilage tissue of the affected joints more actively occurred in the group of rats where the course of laser therapy and drugs was applied.

**Conclusion.** Thus, changes in biochemical markers of connective tissue status in rats during experimental knee joints osteoarthritis indicated a higher efficiency of the treatment regimen with the use of low-intensity infrared laser radiation compared with drug only therapy. We used the infrared laser radiation complex and glucosamine hydrochloride and sodium diclofenac in conservative treatment of rats with experimentally induced knee joints osteoarthritis after 24 days of treatment according to the above-mentioned scheme. As a result we observed a significant decrease in the content of glycoproteins (28.5%), chondroitin sulfates (26.1%), alkaline phosphatase activity (32.3%), as well as I and II GAG fractions, namely chondroitin-6 (28.7%) and chondroitin-4-sulfate (40.3%). Moreover, the studied markers indicators approached the level of the same indicators in the intact animals. The obtained results of the experimental study on rats showed that the use of laser therapy in the early stages of knee joints osteoarthritis can significantly improve the clinical condition of patients, and be effective in the treatment of knee joints osteoarthritis on early stages.

### References

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