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ORIGINAL PAPER

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Intraarticular administration of chondroitin sulfate in experimental osteoarthritis

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ABSTRACT

Introduction and aim. Osteoarthritis (OA) is generally a progressive disease that affects synovial joints, resulting in abnormalities to articular cartilage subchondral bone, synovium, and adjacent soft tissues. The purpose of this work was to investigate the specific activity of chondroitin sulfate (CS) in intra-articular and intramuscular administration to laboratory rabbits in experimental OA.

Material and methods. OA was induced in rabbits by a single injection of mono-iodoacetate in knee joint. CS was administered intra-articularly and intramuscularly. The analysis of biochemical markers and macroscopic assessment of rabbit knee joints was performed.

Results. Intramuscular and intra-articular injection of CS reduces the intensity of the degenerative-dystrophic process due to the impact on inflammatory and the activation of anabolic mechanisms. Intra-articular administration of CS leads to a greater increase in the level of factors of bone and cartilage formation and a greater decrease in the levels of factors of the acute phase of inflammation and factors that destroy the cartilage matrix.

Conclusion. Intramuscular administration of CS revealed a lower intensity of destructive changes in the cartilaginous surface of the knee joint, and intramuscular – the absence of cartilage destruction and defects of the cartilaginous surface, which indicates the peculiarity of the topical effect of the CS.

Keywords. chondroitin sulfate, chondroprotection, intra-articular and intramuscular administration, osteoarthritis

Introduction

Osteoarthritis (OA) is traditionally characterized as a heterogeneous group of chronic degenerative-dystrophic, and more recently inflammatory diseases of the joints and spine of various etiologies, but with similar progressive morphological changes of cartilage, subchondral bone, and synovial capsule, which lead to a decrease in the functional activity of patients, the ability to self-care and disability of patients.[1-5]

The social significance of OA is due to the fact that the first signs or manifestations of OA may be at the age of 30, and with age, there is an increase in the incidence of the disease. It is established that the development of OA increases from 2 to 10 times at the age from 30 to 65 years. According to the WHO, 80% of the population aged 50-60 suffer from OA, more than half of them have limitations in daily life activities, and 25% ‑ can not cope with basic daily responsibilities. Everyone under the age of 80 suffers from OA. In addition, patients with OA make up 30% of patients with disabilities due to diseases of the musculoskeletal system.[6,7]

The cause of OA is considered to be a violation of the mechanical load on the joint and the possibility of physiological recovery of hyaline cartilage after this

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load, which leads to the formation of a «vicious circle» associated with the development and maintenance of inflammation in the joint and progressive cartilage destruction.[8-10]

It is known that the main symptom that aggravates the course of OA and causes disability is pain.[1,4,5] To date, the cause of joint pain in OA has not been definitively established.[11,12] It is believed that the answer to the question of the cause of joint pain in OA can be found in the study of molecular biology and biochemistry of OA. From this point of view, OA is a complex process, the reversibility of which depends on the action of the restorative and regenerative processes in the joint by affecting chondrocytes in the direction of anti-inflammatory and chondroprotective action.[13,14]

Treatment of OA is one of the most pressing problems of modern medicine around the world, not only because this pathological condition is very common, characterized by severe course and consequences that lead to disability, but also because of the lack and inconsistency of data on the effectiveness of various measures and drugs. In this aspect, the problem of analgesia and the impact on the structural organization of articular cartilage with the help of drugs of the group Symptomatic Slow-Acting Drugs in Osteoarthritis (SYSADOAs).[15,16]

At present, it is believed that the mechanical properties of articular cartilage depend on the structural organization of the cartilage matrix, namely the interaction of water molecules and macromolecules – collagen, proteoglycans, and non-collagenous proteins.[17,18] The synthesis of matrix macromolecules is determined by the functional activity of chondrocytes, which decreases with age, leading to disruption of the normal ratio of components of articular cartilage and the progress of its degeneration, and disruption of subchondral bone remodeling contributes to the degradation of cartilage tissue. Approaches to conservative treatment with SYSADOAs, PRP-, and SVF-therapy, which are aimed at restoring the cellular composition of cartilage, stimulating the regenerative potential of the joint as a whole, and eliminating the main manifestations of OA, are based on this.[19,20]

Some authors emphasize the importance of the meniscus into the OA knee joint as the function of the meniscus is very important to make the joint surfaces concordant and to better cushion the load during movement and the role of some biomarkers such as lubricin.[21,22]

Aim

The purpose of this work was to investigate the specific activity of chondroitin sulfate (CS) in intra-articular and intramuscular administration to laboratory rabbits in experimental osteoarthritis (OA).

Material and methods

Design

Experimental studies were performed on nonlinear, healthy, adult 16 Chinchilla rabbits (8 males and 8 females) weighing 2.5 kg. The animals were on a standard diet and in standard vivarium conditions (air temperature: 22±2°C, light/dark cycle: 12/12 hours) in accordance with sanitary and hygienic standards and received food and water ad libitum.[23,24]

Ethics approval

The Committee on Bioethics of the Oles Honchar Dnipro National University approved the study protocol and all procedures related to the maintenance of the animals, their humane treatment, and their use in the experiments. These also complied with Good Laboratory Practice requirements and the European Convention for the Protection of vertebrate animals used for experimental and other scientific purposes.

Osteoarthritis model

Groups 2, 3, and 4 of rabbits had osteoarthritis induced by mono-iodoacetate (MIA). The MIA osteoarthritis model involved a single MIA injection (3 mg in 50 μL of sterile saline) into the knee joint, as described by Guingamp et al.[25-27]

Experimental groups

After the formation of the experimental model of OA on the 28th day of the experiment, the rabbits were divided into experimental groups as follows:

Group 1: Intact animals (4 rabbits);

Group 2: OA without «treatment» (4 rabbits);

Group 3: OA + intramuscular administration of CS (4 rabbits);

Group 4: OA + intra-articular administration of CS (4 rabbits).

Chondroitin sulfate administration

CS was ARTRIDA® (manufacturer by «Haupt Pharma Livron», France and promotion by Lithuanian pharmaceutical company «Farmlyga»).

CS was used intra-articularly 0.24 ml 1 time in 3 days 5 times on 28, 31, 34, 37, and 40 days of the experiment and intramuscularly – 0.24 ml 1 time per day every other day for 25 days (from 28 days of the experiment to 53 days).

Biochemical parameters

On the 28th day of the experiment (this period corresponds to the maximum period of formation of the experimental model OA), blood was taken from animals of all experimental groups to assess the level of biochemical markers and animals 3 and 4 groups began administration of CS according to the above scheme.

On days 43 and 53 of the experiment (depending on the method of CS administration) blood was taken from animals of groups 3 and 4 to assess the level of biochemical markers.

The test systems of the following markers manufactured by «MyBioSource» (San Diego, CA, USA) and Elabscience Biotechnology Co., Ltd. (Wuhan, China) were used in the study:

1. Markers of cartilage and bone destruction: C-telopeptide of collagen type 2 (CTX-II) and C-telopeptide of collagen of type 1 (CTX-I) reflect the degree of destruction of cartilage and bone tissue, respectively. Relevant markers are found in the serum due to the destruction of cartilage and bone tissue and the breakdown products of collagen types 1 and 2 into the blood.

2. Markers of enzymes that destroy the cartilage matrix: matrix metalloproteinases (MMP) 3, 9, and 13 reflect the «state» of the cartilage matrix.

3. Markers of bone formation: bone alkaline phosphatase (BALPL) reflects the intensity of anabolic processes in bone tissue. Allows assessing the regenerative potential of bone tissue.

4. Biochemical markers of inflammation (acute serum inflammation proteins): C-reactive protein (CRP), interleukins (IL) 1, 6, and 8, tumor necrosis factor-alpha (TNF-α). Reflect the body's overall response to inflammation. Are non-specific markers of inflammation.

Blood samples were taken from the marginal ear vein of rabbits after fasting for 12 hours. Blood samples were left to coagulate for 2 hours at 4°C. Then they were centrifuged at 4000 rpm for 15 min at 4°C to obtain serum. Serum samples were aliquoted in 0.5 ml tubes and stored at -80°C until analysis.

Serum concentrations of cartilage and bone markers, pro-inflammatory/anti-inflammatory cytokines were measured by quantitative solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) using rabbit-specific test kits and following the manufacturer's instructions.

The procedure for studying biochemical markers was performed as follows. To each well of the plates was added a standard solution or serum sample (100 μL), the plate was covered and incubated for 2 hours at 37°C. Then the liquid from each well was removed and added a working solution of biotinylated antibody (100 μL), specific to the corresponding marker, the content of which is examined in the serum of rabbits. The plate was covered and incubated for 1 hour at 37°C. The wells were washed three times with wash buffer (350 μL), avidin conjugated with horseradish peroxidase (100 μL) was added to each well, the plate was covered and incubated for 30 minutes at 37°C. The wells were washed five times with wash buffer (350 μL), a tetramethylbenzidine substrate solution (90 μL) was added to each well, and the plate was incubated in the dark for 15-30 minutes at 37°C. Subsequently, a stop solution (50 μL) was added to each well. The optical density of each well was determined using a Rayto RT-2100C microplate reader (Rayto Life and Analytical Sciences Co., Ltd., China) at 450 nm. The concentration of the test marker was calculated by comparing the optical density of each sample with a standard 4-parameter logistics curve constructed using AssayFit Pro 1.31. Each sample was tested twice according to the test system manufacturer's instructions.

Sectioning and macroscopic parameters

All animals were killed accordind to Ethical Approvement by intraperitoneal administration of a thiopental sodium solution (40 mg/kg body weight) and samples of knees were taken.[28] Rabbits of group 4 were killed on day 43, and rabbits of groups 1, 2, and 3 were killed on day 53.

Macroscopic evaluation of Chinchilla rabbit joint tissues was performed using generally accepted standard methods and surgical instruments.

Statistical analysis

Physical parameters are reported as means $\pm$ errors. Depending on the normality of the distribution (as assessed using the Shapiro-Wilk test) and the groups being compared, the Student's t-test, the paired t-test, the Mann-Whitney U-test, or the paired Wilcoxon test were generally used. For knee circumference, a one-way dispersion analysis and Duncan's test were used. The level for significance was taken to be $P<0.05$. Statistical processing was performed using STATISTICA 6.1 software product provided (StatSoft Inc., serial No AGAR909E-415822FA).

Results

Macroscopic parameters

At the research of tissues of a knee joint at animals of group 1, the normal structure of a joint is defined (Fig. 1). Cartilage has a white, bright color. The cartilage surface is shiny, without pathological usurpations and defects. The capsule of the joint is not thickened the usual color. There are no infiltrative and inflammatory phenomena around the cartilaginous formations. Pathological changes in the structure of the knee joint and soft tissues have not been identified.

At the research of tissues of a knee joint at animals of group 2, the expressed pathological changes (Fig. 2) are defined. The cartilage has a yellow, dull color. The cartilage surface is heterogeneous, focal pathological usurpations and defects are determined. The capsule of the joint is thickened, hyperemic, has a pronounced capillary and vascular network. Infiltrative and

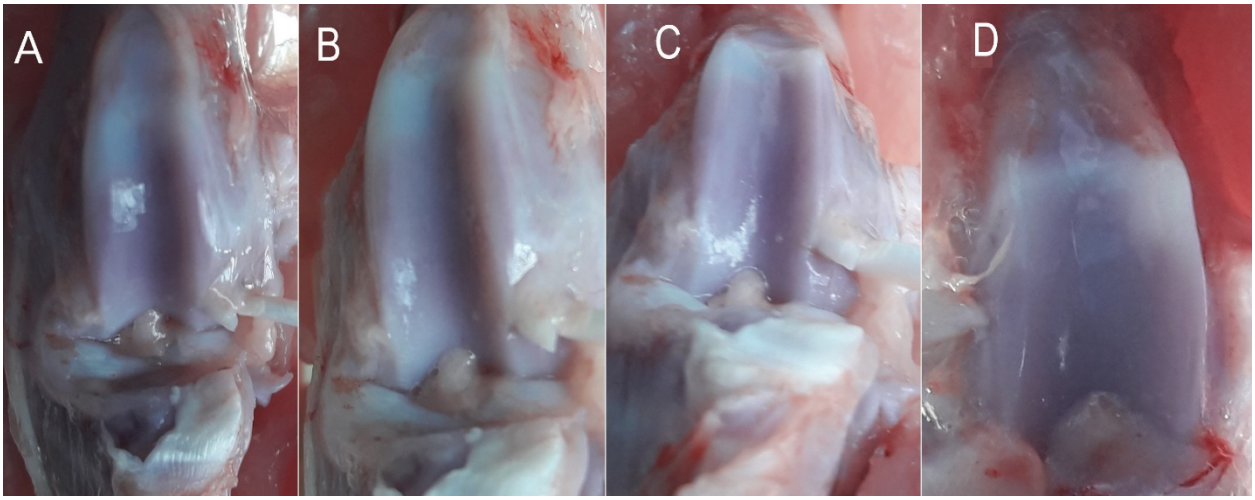

Fig. 1. Macroscopic structure of the tissues of the knee joint of rabbits of group 1 (the distal part of the femur of the rabbit is shown)

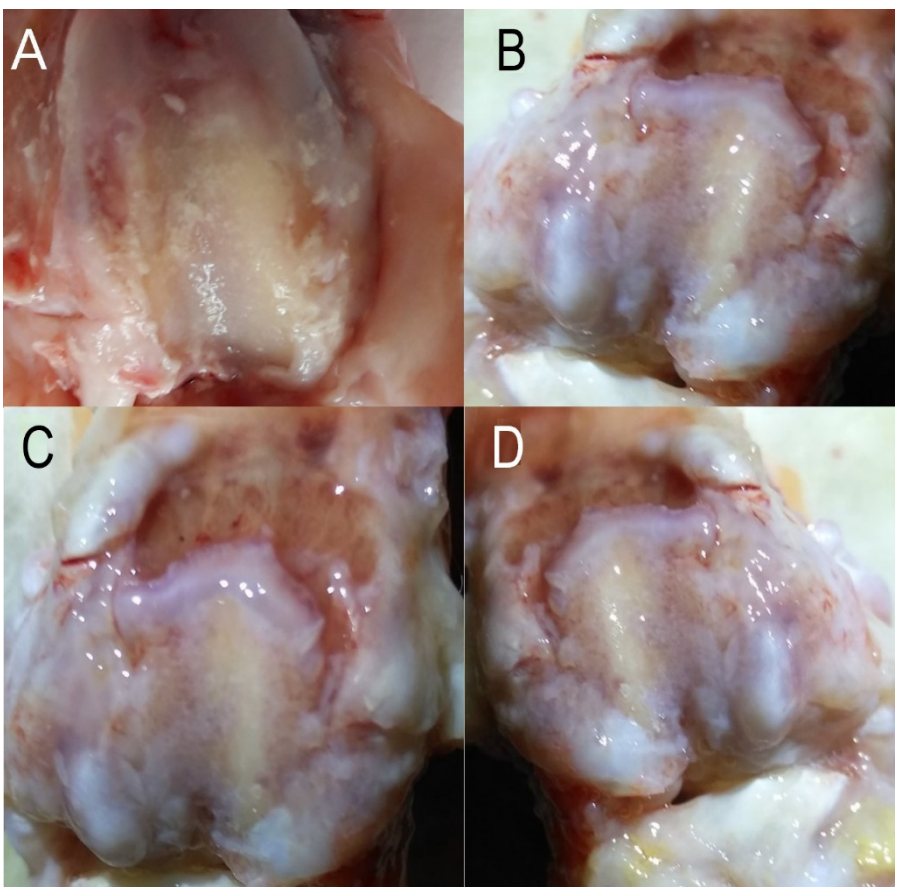

Fig. 2. Macroscopic structure of the tissues of the knee joint of rabbits of group 2 (the distal part of the femur of the rabbit is shown)

inflammatory phenomena around bone and cartilage formations are determined. The identified phenomena confirm the adequacy of the simulation of OA of the knee joint in animals using MIA.

At the research of tissues of a knee joint at animals of groups 3, pathological changes are defined (Fig. 3A-D). The cartilage has a yellow, bright color. In Fig. 3A-B cartilage surface is heterogeneous, individual focal pathological usurpations and defects are identified. In Fig. 3D the cartilage surface is homogeneous, pathological usurpations and defects are not defined. The capsule of the joint is slightly thickened, hyperemic, in Fig. 3A has a pronounced capillary and vascular network. Infiltrative and inflammatory phenomena around the cartilaginous formations are moderate.

At the research of tissues of a knee joint at animals of groups 4, moderate pathological changes (Fig. 4A-D) are defined. The cartilage has a yellow, bright color.

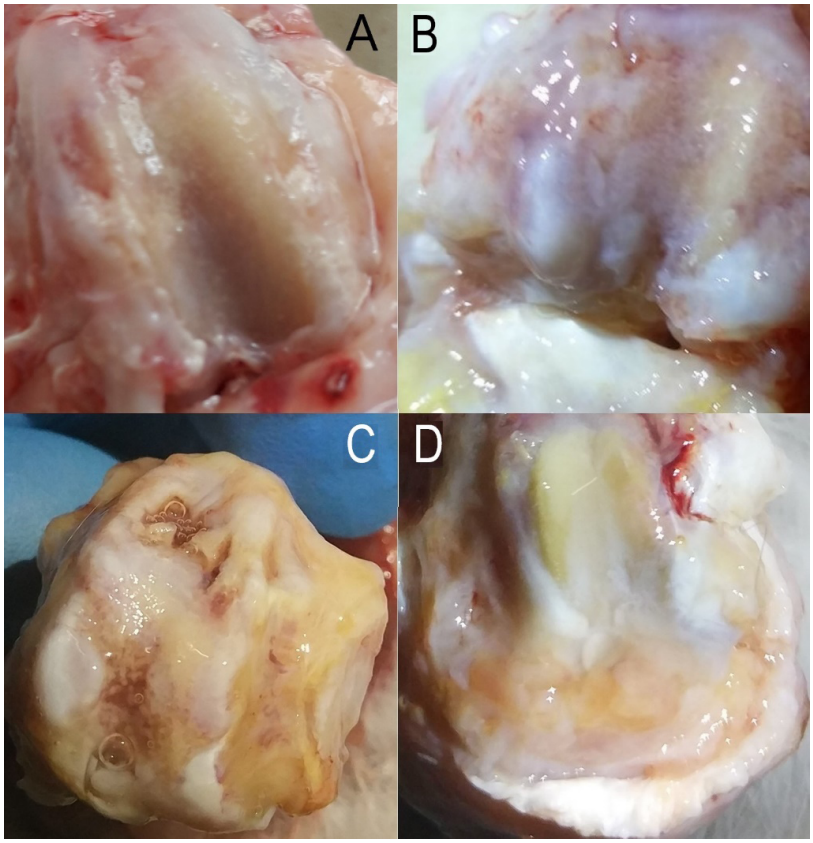

Fig. 3. Macroscopic structure of the tissues of the knee joint of rabbits of group 3 (the distal part of the femur of the rabbit is shown)

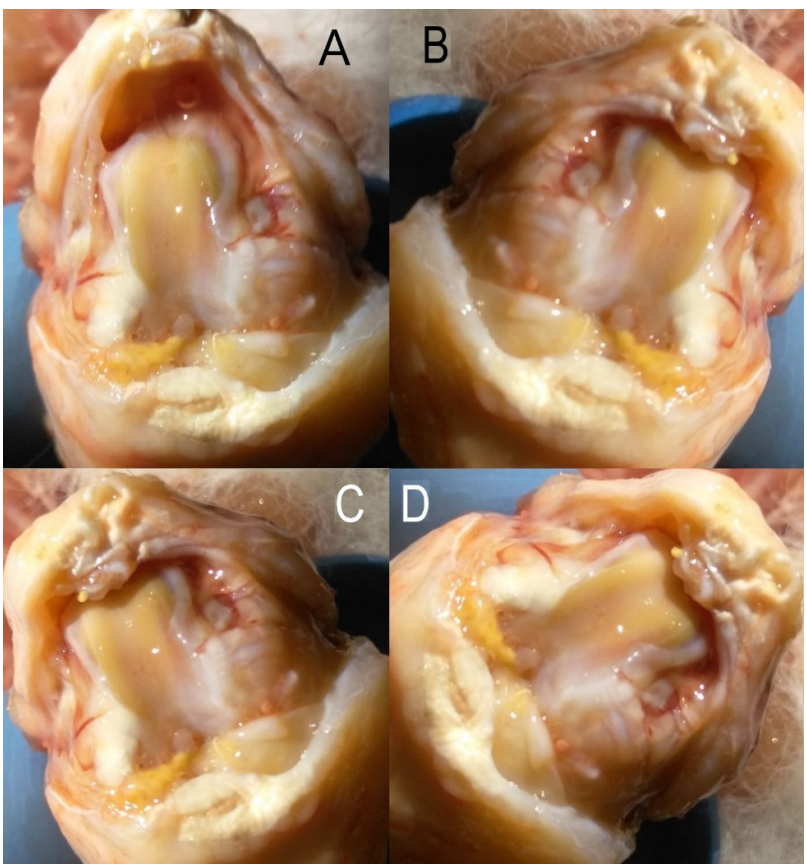

Fig. 4. Macroscopic structure of the tissues of the knee joint of rabbits of group 4 (shown rabbit knee joint intersected along with the joint space)

Table 1. The average values of biochemical parameters in animals of the experimental groups at 28 days (M)

	TNF-α, pg/mL	BALPL, ng/mL	MMP-3, ng/mL	MMP-13, ng/mL	CRP, pg/mL	IL-6, pg/mL	IL-8, pg/mL
Group 1 (n=4)	0	2.66#	**0**	**0**	0	**0**	**0**
Group 2 (n=4)	44.70*	7.87*	3.88*	9.0*	642.50*	68.44*	931.76*
Group 3 (n=4)	41.08*	7.95*	4.16*	10.94*	753.59*	77.86*	968.81*
Group 4 (n=4)	44.35*	7.16*	4.42*	9.69*	581.19*	69.42*	912.64*

*p<0.05 versus Group 1

#p<0.05 versus Group 2

0 – marker is not determined in the blood sample

Table 2. The average values of biochemical parameters in animals of the experimental groups for 43 days (M)

	TNF-α, pg/mL	BALPL, ng/mL	MMP-3, ng/mL	MMP-13, ng/mL	CRP, pg/mL	IL-6, pg/mL	IL-8, pg/mL
Group 1 (n=4)	0	2.63#	**0**	**0**	**0**	**0**	**0**
Group 2 (n=4)	32.45*	7.88*	5.68*	8.90*	590.86*	122.01*	524.35*
Group 3 (n=4)	–	–	–	–	–	–	–
Group 4 (n=4)	15.78*#	14.33*#	2.72*#	4.22*#	110.77*#	35.01#	186.35*#

*p<0.05 versus Group 1

#p<0.05 versus Group 2

0 – marker is not determined in the blood sample

– marker definition was not performed

Table 3. The average values of biochemical parameters in animals of the experimental groups for 53 days (M)

	TNF-α, pg/mL	BALPL, ng/mL	MMP-3, ng/mL	MMP-13, ng/mL	CRP, pg/mL	IL-6, pg/mL	IL-8, pg/mL
Group 1 (n=4)	0	2.07#	**0**	**0**	**0**	**0**	**0**
Group 2 (n=4)	29.62*	6.32*	4.07*	7.78*	754.32*	120.71*	541.11*
Group 3 (n=4)	18.55*	14.11*#	2.93*	4.08*	104.24*#	36.30*#	193.76*#
Group 4 (n=4)	–	–	–	–	–	–	–

*p<0.05 versus Group 1

#p<0.05 versus Group 2

0 – marker is not determined in the blood sample

– marker definition was not performed

The cartilage surface is homogeneous, focal pathological usurpations and defects are not defined. The capsule of the joint is normal, the vascular pattern is not determined. Infiltrative and inflammatory phenomena around the cartilaginous formations are not defined.

Biochemical parameters

The obtained results are presented in Tables 1, 2, and 3. The average values (M) of biochemical parameters on the 28th day of the study in animals of the experimental groups are presented in the Table 1.

The average values (M) of biochemical parameters on the 43rd day of the study in animals of the experimental groups are presented in the Table 2.

The average values (M) of biochemical parameters on the 53rd day of the study in animals of the experimental groups are presented in the Table 3.

Analysis of the results (Table 1-3) suggests that on the 28th day of the experiment in groups 2, 3, and 4 there is an increase in levels of TNF-α, BALPL, MMP-3, MMP-13, CRP, IL-6, and IL- 8 in relation to animals of group 1 (intact), which reflects the activity of the pathological process caused by the action of MIA on cartilage tissue. Levels of markers TNF-α, BALPL, MMP-3, MMP-13, CRP, IL-6, and IL-8 in group 2 animals (without treatment) on 28, 43, and 53 days of the experiment reflect a gradual decrease in the intensity of inflammatory and destructive processes in knee joints of rabbits, which corresponds to the peculiarities of OA in the experimental reproduction of the degenerative-dystrophic process by intra-articular administration of MIA.

On day 43 of the study, high levels of TNF-α, MMP-3, MMP-13, CRP, IL-6, and IL-8 were observed in groups 2 with respect to groups 1 and 4, reflecting the further development of OA. However, in group 4 there is a decrease in TNF-α, MMP-3, MMP-13, CRP, IL-6, and IL-8 relative to group 2, which corresponds to a decrease in the intensity of catabolic and inflammatory processes, and an increase in BALPL indicates a more

pronounced intensity anabolic process in bone and cartilage.

Almost a similar pattern is observed on the 53rd day of the experiment. In group 2, high levels of markers TNF-α, MMP-3, MMP-13, CRP, IL-6, and IL-8 were observed relative to groups 1 and 3, which reflects the further development of OA. At the same time, in group 3 there is a decrease in TNF-α, MMP-3, MMP-13, CRP, IL-6, and IL-8 relative to group 2, which corresponds to a decrease in the intensity of catabolic and inflammatory processes, and an increase in BALPL indicates the more pronounced intensity of anabolic processes in bone and cartilage.

Noteworthy is the change in BALPL levels in group 4 on day 43 of the study and in group 3 on day 53 of the experiment, which reflects the intensity of anabolic processes in the knee joints of rabbits. These values are 14.33 and 14.11 ng/mL, respectively. When comparing the obtained values with the indicators of group 2 (without treatment) there is an increase in the intensity of anabolic processes 1.9 and 2.23 times, respectively (Tables 2 and 3).

Analyzing the dynamics of changes in the levels of markers TNF-α, BALPL, MMP-3, MMP-13, CRP, IL-6, and IL-8 in group 3 (intramuscular administration) and group 4 (intraarticular administration) can be argued that intramuscular and intraarticular administration of the CS leads to a decrease in the intensity of the degenerative-dystrophic process due to the impact on inflammatory phenomena and activation of anabolic mechanisms.

A comparison of the results obtained in the group 3 (intramuscular administration) and the group 4 (intra-articular administration) suggests that intra-articular administration of CS leads to a greater increase in BALPL and a greater decrease in markers TNF-α, MMP-3, IL-6, and IL-8. However, no statistically significant reliability was obtained between these indicators of groups 3 and 4 (Tables 2 and 3).

Discussion

When comparing the results obtained between the experimental groups of animals, a sharp difference in macroscopic changes with group 1 is determined, which indicates the presence of degenerative-dystrophic changes in the knee joints of animals of groups 2, 3, and 4. The most pronounced intensity of pathological changes was determined in animals of group 2, which confirms the adequacy of the performed OA model. When comparing macroscopic changes between groups 2 and 3, a lower intensity of destructive changes in the cartilaginous surface of the knee joint was determined. When comparing macroscopic changes between groups 2 and 4, the absence of pathological usurpation and cartilaginous surface defects was determined.

Thus, based on the obtained results, it can be stated that the intramuscular administration of CS revealed a lower intensity of destructive changes in the cartilaginous surface of the knee joint, and intra-articular – the absence of pathological usurpation and cartilage surface defects, indicating the topical effect of CS. In our opinion, the obtained results may depend on the CS drug used. We have previously confirmed the importance of using a high-quality pharmaceutically pure active main ingredient of CS to ensure optimal efficacy and safety of the final product in patients with osteoarthritis.[29]

In the process of biochemical studies, no indicators of CTX-I, CTX-II, IL-1, and MMP-9 were determined due to the fact that the level of studied markers in blood samples was less than the sensitivity of test systems, which does not contradict the literature and reflects study performed.[30-34] However, low levels of CTX-I and CTX-II indicate the absence of severe destructive changes caused by the introduction of MIA, which corresponds to the initial manifestations of OA and adequate conditions for the appointment of the CS.[35-37]

Limitations

The author did not provide some morphological information and related biomarkers expression through histology, histochemistry, and immunohistochemistry techniques.

Conclusion

To date, there is an open discussion about the appropriateness and effectiveness of CS for OA. There are different opinions on these issues, sometimes of the opposite nature. There is also no consensus in the current literature about the effectiveness of intra-articular injection of CS in OA. In our study, we tried to visualize morphological and biochemical changes during intramuscular and intra-articular administration of CS in experimental OA.

We found that intramuscular administration of CS revealed a lower intensity of destructive changes in the cartilaginous surface of the knee joint, and intra-articular - the absence of pathological usurpation and cartilage surface defects, indicating the topical effect of the CS. Intramuscular and intra-articular administration of CS reduces the intensity of the degenerative-dystrophic process due to the impact on inflammatory phenomena and the activation of anabolic mechanisms. However, intra-articular administration of CS leads to a greater increase in the level of markers of bone and cartilage formation and a greater decrease in the levels of markers of the acute phase of inflammation and markers that destroy the cartilage matrix.

Declarations

Funding

The study was performed within the project «Preclinical study of the specific activity of the drug ARTRIDA® solution for injection, manufactured by Haupt Pharma Livron (France) with intra-articular and intramuscular administration to laboratory rabbits» (customer CJSC «Farmlyga» *Republic of Lithuania*)

Author contributions

Conceptualization, D.N.; Methodology, D.N.; Software, D.N.; Validation, D.N.; Formal Analysis, D.N.; Investigation, D.N.; Resources, D.N.; Data Curation, D.N.; Writing – Original Draft Preparation, D.N.; Writing – Review & Editing, D.N.; Visualization, D.N.; Supervision, D.N.; Project Administration, D.N.; Funding Acquisition, D.N.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

The Committee on Bioethics of the Oles Honchar Dnipro National University approved the study protocol and all procedures related to the maintenance of the animals, their humane treatment, and their use in the experiments. These also complied with Good Laboratory Practice requirements and the European Convention for the Protection of vertebrate animals used for experimental and other scientific purposes.

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