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Histological changes in the joint tissues of rabbits with gonarthrosis

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Osteoarthritis is a chronic degenerative disease that is accompanied by a progressive degeneration of the articular cartilage, remodeling of the subchondral bone, development of a chronic inflammation of the synovial sheath, and damage to the ligamentous apparatus. In this study, we analyzed the histological changes in the main structures of the ankle joint of the rabbits (*Oryctolagus cuniculus domesticus* Linnaeus, 1758, Leporidae), namely: joint cartilage, subchondral bone, synovial sheath, ligamentous apparatus, and menisci on days 7, 14, 21, 28, and 35 after experimentally modeling osteoarthritis. To model the experimental osteoarthritis, we chose the method of intra-articular injection of 4% solution of retinyl acetate and kojic acid (Yellow peel, Medicare, Germany) in a dose of 0.7 mL. The intra-articular injections were performed twice using 23G needles (0.6 * 32 mm) with a seven-day interval. In the earlier stages of development of the pathological state (day 7 of the study), we observed the beginning of degeneration of the joint cartilage, with a local decrease in proteoglycans, which are an important component of the intercellular material of the connective tissue. Furthermore, we detected a slight thickening of the areas of the subchondral bone and a moderate inflammatory infiltration of the joint's synovial sheath. On day 14 of the studies, we observed a progressing thinning of the articular cartilage with a disturbance of the structure of chondrocytes, osteosclerosis of the subchondral bone, and an active angiogenesis in the synovial sheath. On day 21 of the study of the pathological changes, we observed deep fissures in the cartilage, the development of osteophytes, and an enlargement of the cavities in the subchondral bone, whereas the synovial sheath was undergoing a chronic form of the inflammation processes with fibrotic changes. On day 28, we observed an almost complete breakdown of the joint cartilage, significant osteosclerosis, a notable formation of osteophytes, and total fibrotic changes in the regions of synovial sheath of the affected ankle joint. Additionally, we decided to conduct studies on day 35 to gain an understanding of the further course of the pathology. In this period, we found that the osteosclerosis pathogenesis in the rabbits following the use of the indicated drug happened unevenly. The obtained results confirmed the dynamic and systemic character of degenerative-inflammatory processes in the ankle joint of the rabbits with induced osteoarthritis. This study offers insights into the pathogenesis of osteoarthritis and can contribute to the development of effective therapeutic approaches.

Keywords: osteoarthritis; histological changes; morphology; articular cartilage; orthopedic pathology; retinyl acetate; subchondral bone; ankle joint.

Introduction

Osteoarthritis (OA) is one the commonest pathologies of the locomotor apparatus in animals. Notably, OA is observed in small and large animals, in particular, dogs, cats, horses, and agricultural animals. It is a chronic degenerative disease, which is characterized by breakdown of the articular cartilage, remodeling of the subchondral bone, degeneration of the synovial sheath, and formation of osteophytes, thereby leading to joint ache and limited mobility of the animals.

Osteoarthritis makes up around 80% of cases of limpness and joint diseases in companion animals. Despite this fact, this pathology is usually associated with animals of older age, as indicated by a recent report on the prevalence of osteoarthritis and respective clinical signs in young dogs (aged 8 months to 4 years). According to the study, 39.8% of the animals exhibited radiographic features of osteoarthritis at least in one joint. Furthermore, 23.6% of the dogs had clinical signs of osteoarthritis, which was confirmed by X-ray images, and also pain syndrome in the joint. This means that in available reports, the actual prevalence of OA can be underrepresented (Alves et al., 2024).

From September 2023 to August 2024, at the scientific laboratories of the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine (Kyiv) and the veterinary clinic Shanty (Kyiv), we examined dogs with different pathologies of the locomotor apparatus. Total of 52% of the examined dogs had osteoarthritis; 43% of the animals were diagnosed with disruption of the cruciate ligament; 1.2% of the dogs were observed to have bruises of various degrees of severity; and 0.6% and 2% of the cases accounted for joint dislocations and foreign bodies, such as osteophytes and arthroliths (joint mice), respectively. The assessment of the clinical condition of the dogs revealed that the degenerative-dystrophic disease of the ankle joint that occurs due to gradual physical degeneration of the joint cartilage was accompanied by pain symptoms in all the animals (Gorkava et al., 2024).

The studies by Moritz Roitner et al. found that the prevalence of osteoarthritis (OA) in the shoulder, elbow, hip, and ankle joints was higher in dogs aged over eight years, with orthopedic complaints serving as the primary clinical manifestation. The dogs took part in the study if the X-rays included one or several abovementioned joints. The X-rays were analyzed by three different examiners and evaluated according to the degree of severity. The prevalence of OA accounted for 39.2%, 57.4%, 35.9%, and 36.4% in the shoulder, elbow, hip, and ankle joints, respectively. It should be noted that a higher OA prevalence was observed in the heavier dogs. Sex and castration of the animals were not factors to OA (Roitner et al., 2024).

Limited by ethic expertise, some programs of research on ankle osteoarthritis cannot be realized, although experimental studies on animal models can additionally reveal the pathogenesis and point to methods of treatment. In recent years, some animal models of OA were broadly used for studying the ways in which the disease develops and for preclinical testing of new methods of treatment (Song et al., 2024).

The studies provided a better understanding of complex mechanisms, including inflammatory, metabolic, and post-traumatic processes that can lead to the disease. Moreover, knowledge of the pathophysiology of pain helps outlining mechanistic targets (Tang et al., 2025).

That is why research on histological changes in the tissues of the ankle joint of rabbits using experimental osteoarthritis is key for a deeper understanding of the disease's pathogenesis, development of effective methods of early diagnostics, and improvement of therapeutic strategies. Thanks to clinical studies on osteoarthritis, the quality of life of the veterinary patients has been substantially improved. Such studies have introduced new approaches to treatment, such as regenerative medicine, use of chondroprotectors, and physiotherapy.

Analysis of changes in the cartilage, subchondral bone tissue, and also synovial sheath allows assessing the mechanisms through which the joint is destroyed during different stages of pathological process.

Studies on histological markers of degeneration, inflammatory reactions, and remodeling of the ankle tissue can foster improvements of the methods of early diagnostics of osteoarthritis. Furthermore, the efficacy of new therapeutic strategies can be assessed. Therefore, studying the structural changes in the tissues of the rabbit's joint is a relevant task of modern morphology, pathophysiology, and experimental medicine.

The ankle joint is one of the most heavily loaded anatomical structures in rabbits, which makes it vulnerable to degenerative changes, such as osteoarthritis (OA). Histological analysis of the tissues of the ankle joint allows evaluating key pathological processes, including degeneration of the cartilage, remodeling of subchondral bone, inflammatory changes in the synovial sheath, and formation of osteophytes (Olifirenko & Savosko, 2021).

The ankle of rabbits is a complex synovial articulation that includes the joint cartilage, subchondral bone, synovial sheath, ligamentous apparatus, and menisci. Osteoarthritis (OA) causes structural and functional changes in all these components, which progress over time. Each histological structure undergoes specific pathological changes, depending on the stage of the disease (Olifirenko & Savosko, 2021).

It is crucial for us to document the transition from the norm to pathology, but for this purpose, we must first understand and demonstrate what the normal state is. Many researchers consider studies on rabbits expedient, but whether we can compare the loads on the joints of different animal species remains a topic of discussion (Alan et al., 2021).

Osteoarthritis is a chronic degenerative disease that is characterized by a progressing degeneration of the cartilaginous tissue, remodeling of the subchondral bone, development of plica syndrome, and formation of osteophytes (Büşra Kibar Kurt, 2023; Yu, 2025). In rabbits (Oryctolagus cuniculus), osteoarthritis often affects the ankle joint, which is due to heavy mechanical loading and the joint's complex structure.

The Osteoarthritis Research Society International, OARSI, has defined osteoarthritis (OA) as a disorder that affects movable joints, which first manifests as an abnormal metabolism in the joint tissue, and then as functional disorders that often lead to the disease (Kraus et al., 2015). Thus, OA is considered as a common chronic degenerative form of arthritis that affects the synovial joints of people and different species of animals (Cope et al., 2019).

In 2023, the prevalence of OA in cats ranged 16% to 91%, depending on the studied population, according to the statistical data of the researchers of the Warsaw Institute (Bonecka et al., 2023). Osteoarthritis is common in dogs of larger breeds, accounting for 85% of the general number of the animals with "chronic" limpness examined in 2023 (Gorkava et al., 2023).

Ankle osteoarthritis develops as a result of disbalance between destruction and regeneration of the cartilage. The main pathophysiological processes include degeneration of the cartilage (decrease in the number of proteoglycans, breakdown of collagen fibers in the cartilaginous tissue, and formation of fissures in the cartilage that deepen to the subchondral bone); presence of synovial inflammation (chronic activation of synodial cells that produce anti-inflammatory mediators, such as interleukin-1β and tumor necrosis factor-α, which accelerate the destruction of the cartilage); remodeling of the subchondral bone (formation of osteophytes, thickening of the subchondral bone and development of microcavities); and derangements in the joint mechanics (changes in the biochemical properties of the ankle joint, which intensify the degenerative processes). Studies on the dynamics of histological changes in the ankle joint in rabbits suffering osteoarthritis in different stages shed light on of the pathogenesis of this disease. The obtained results help in determining the stages of pathological process and their effects based on the joint's main structures: joint cartilage, subchondral bone, and synovial sheath (Szponder et al., 2023; Strafun et al., 2025; Yu et al., 2025).

Immune cells infiltrate the tissues of joints in different stages of the disease, and therefore different cells of the joints release cytokines and chemokines, after which the complement system activates. Soon, factors that affect the destruction of the cartilage are released, such as matrix metalloprotein and prostaglandin E2, leading to a metabolic disbalance of extracellular matrix and irreversible destruction of the cartilage (Emami, 2023).

Available options of treating OA are limited. The disease is hard to treat due to the incomplete understanding of molecular processes and pathways related to this pathology and also limited properties of cartilage regeneration. Metabolism of chondrocytes generates energy through glycolysis, oxidative phosphorylation, and other metabolic pathways. Metabolism of chondrocytes can use different substrates in the joint synovial fluid, from simple sugars to amino acids and fatty acids (Miradj et al., 2024).

The objective of the study was to determine the structural histological changes in the tissues of the ankle joint of rabbits with experimentally modeled osteoarthritis.

Materials and methods

The conditions of keeping and feeding the experimental animals, selection of age groups of rabbits, and their withdrawal from the experiment were provided in accordance with generally accepted methods and requirements in compliance with bioethics. The research protocol was reviewed and approved by the local ethics committee of the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine (Kyiv, Ukraine). The study was carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental or other Scientific Purposes (Strasbourg, France, 18 March 1986, ETS No. 123) and the Law of Ukraine On Protection of Animals from Cruelty (Kyiv, February 21, 2006, No. 3447-IV).

The experimental studies were conducted in 2020-2024 at the Department of Veterinary Surgery named after Academician I. O. Povazhenko, the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine (Kyiv, Ukraine). The histological studies were performed at the Department of Anatomy, Histology, and Pathomorphology of Animals named after Academician V. H. Kasianenko, the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine (Kyiv, Ukraine).

In the study, we used 24 clinically healthy male rabbits weighing 2.5 to 2.8 kg. The maintenance conditions in the vivarium corresponded to zoohygienic norms; the rabbits were consistently provided with high-quality, balanced granulated feed (fed twice a day) and had free access to fresh drinking water. The experimental animals had not been vaccinated or treated from ecto- and endoparasites.

All the animals underwent a preliminary examination, which included collecting anamnesis, as well as general physical and detailed orthopedic examinations. The animals were divided into two groups – control and experimental – each consisting of 12 rabbits. The control-group animals received intra-articular injection of isotonic 0.9% solution of NaCl in a dose of 0.7 mL. In the experimental-group animals, experimental osteoarthritis was modeled by intra-articular injection of 4% solution of retinyl acetate and kojic acid (Yellow peel, Medicare, Germany) in a dose of 0.7 mL under general anesthesia. The intra-articular injections were made twice using a 23G needle (0.6 * 32 mm) with a seven-day interval.

The general anesthesia was performed in accordance with the safe anesthesiological protocols ("Rapid Reference to the Exotic Animal Formulary", 2023) and principles of conducting anesthesia according to the guidelines "Principles of Rabbit Anaesthesia for Veterinary Nurses" (Sibbald et al., 2018). In particular, we used Dexdomitor 0.5 (Orion Pharma, Finland) in a dose of 0.05–0.2 mg/kg for sedation

and Propofol-Lipuro 1% (emulsion 10 mg/mL, 20 mL, B.Braun Melsungen AG, Germany) in a dose of 1–2 mg/kg for induction.

During the modeling of experimental osteoarthritis, the animals were regularly monitored. On days 7, 14, 21, 28, and 35 after modeling osteoarthritis, the animals were withdrawn from the experiment by injecting the sodium thiopental solution (Thiopental 1.0, KyiivMedpreparat Ltd., Ukraine). The ankle joint was removed for histological study. The samples were fixed in 10% solution of neutral formalin. After fixation, we isolated the epiphysis of the tibia, the distal epiphysis of the femur, and the capsule and meniscus of the joint. The bone fragments were deminiralized in a 10% solution of formic acid (Nacalai Tesque, Japan) for 10 days. The samples were dehydrated through a series of alcohols using a Leica TP1020 semi-enclosed benchtop tissue processor (Leica Biosystem, Wetzlar, Germany) with gradual increase in concentration, and were engulfed in paraffin (Leica Surgipath Paraplast Regular). From the paraffin blocks, we prepared serial 5 µm-thick sections and placed them on glass slides. Before staining, the microscope slides were deparaffinated and hydrated with distilled water. We performed staining with hematoxylin-eosin (assessment of general morphology of the tissues), and toluidine blue (measuring the concentration of proteoglycans in the cartilage) to evaluate different structures of the body (Gruber, 1999; Kang, 2003).

The histological sections were studied using a Sigeta Biogenic LED Trino Infinity light microscope (Sigeta, China) with an installed Sigeta MDC-560 CCD camera for microscope (Sigeta, China) with multi-magnification for detailing of the structural changes in the cartilage, subchondral bone, and synovial sheath.

The studies included an analysis of the conditions of the joint cartilage, subchondral bone, synovial sheath, and periarticular tissues. The changes were assessed using a semi-quantitative scale OARSI (International Osteoarthritis Research Society) for laboratory animals, namely: measuring the thickness of the joint cartilage and intensity of Safranin O staining for determining the level of proteoglycans; measuring of the density of osteocytes and the area of sclerotic regions; analyzing the cellular infiltration, thickening of the membrane, and the level of fibrosis.

Results

The changes in the histological structures on day 7 included the first features of degeneration of the superficial layer of the cartilage. In particular, we observed a decline in the number of proteoglycans, which was indicated by poorer intensity of Safranin O staining (Fig. 1a). We saw a local loss of chondrocytes in the superficial zone, retention of cells in the middle and deep layers, and also initial features of remodeling and slight thickening of the subchondral lamina. Small microcavities emerged in the bone tissue. In the structures of synovial sheath, we observed hyperplasia of synoviocytes and a moderate infiltration of the tissue by lymphocytes and macrophages (Fig. 1b).

The changes we saw in the intercellular substance in this monitoring period indicated significant disorders in the synthetic function of chondrocytes, which produced noticeably smaller amount of the main component of intercellular matter of the hyaline cartilage. In the red bone marrow of the spongy bone, located between the joint cartilage and the epiphyseal plate, hematopoiesis had almost completely stopped (Fig. 1c). In the same region, intramembranous ossification had stopped completely, as indicated by the complete absence of osteoblasts at the edge of the bone tissue of the spongy bone diploe (Fig. 1c).

In the epiphyseal plate, gaps emerged between the columns of chondrocytes, although the number of such gaps was notably lower compared with the articular cartilage. Such changes indicated the impaired processes of synthesis in the cytoplasm of chondrocytes. However, this disorder was significantly weaker compared with the articular cartilage. Moreover, in this period of observations, we also recorded microscopic changes in the synovial sheath of the ancle joint, which were characterized by its edema and disorganization (Fig. 1d).

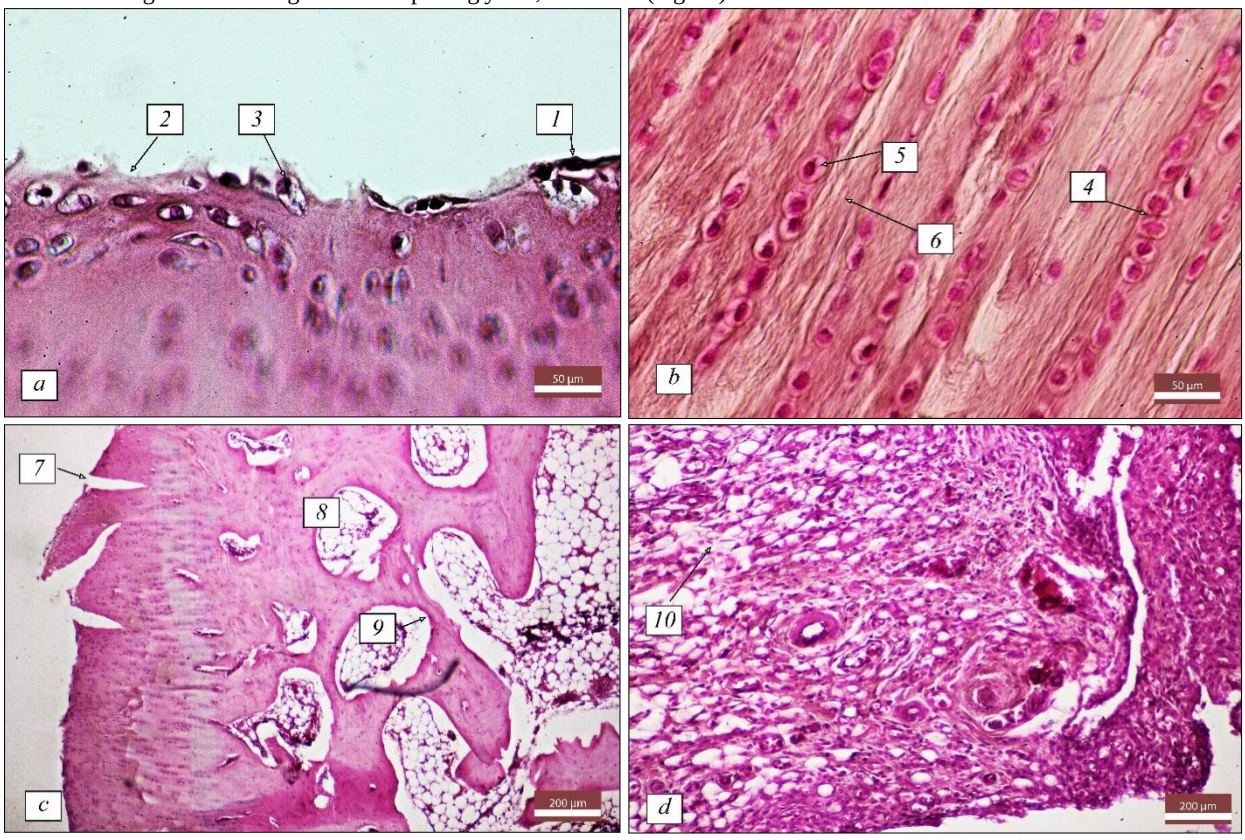

Fig. 1. Structural changes in the tissues of the ankle joint of the rabbit on day 7 after injecting retinyl acetate: *a* – the articular cartilage of the tibia; *b* – the epiphyseal cartilage of the tibia; *c* – the femur epiphysis; *d* – synovial sheath: *1* – perichondrium; *2* – absence of perichondrium; *3* – destruction of chondroblast; *4* – a column of chondrocytes; *5* – oxyphilous chondrocyte cytoplasm; *6* – fibers of the intercellular substance of the cartilaginous tissue; *7* – superficial fissure of the joint cartilage; *8* – absence of hematopoietic cells in the bone marrow; *9* – absence of osteoblasts at the boundary of the bone tissue; *10* – edema and disorganization

The changes on day 14 included: a notable destruction of the superficial layer of the cartilage, with fissures reaching the middle layers; a significant decline in the concentration of proteoglycans throughout the cartilage, hyalinization of some areas of the matrix, and the emergence of vacuolization in chondrocytes (Fig. 2c).

In the subchondral bone, we observed an increase in the density of the subchondral plate as a result of sclerosis and the formation of individual microcavities, which indicate intensification of bone resorption (Fig. 2a); an enhancement of the inflammatory infiltration of the synovial sheath, with an increase in the number of plasmatic cells and macrophages; and also edema and vascularisation.

In the areas of the articular cartilage where fissures were absent, the cartilage was notably thinned (Fig. 2b). In our opinion, the thinning of the joint cartilage resulted from the absence of perichondrium and destruction of chondroblasts, as confirmed histologically on day 7. These changes hindered the formation and repair of the joint cartilage. In most areas of the thinned joint cartilage, either the perichondrium was absent, or there was delamination of large regions from the underlying cartilaginous tissue (Fig. 2b).

Changes in the spongy bone tissue, located between the articular cartilage and epiphyseal plate, were similar to those on day 7. The epiphyseal plate in this period of observations was becoming uneven (Fig. 2c). The matrix of the cartilaginous tissue in most areas was unevenly stained, and chondrocytes in many areas had no organized arrangement – they did not form columns typical of the epiphyseal plate, and were randomly dispersed across the matrix. The latter contained a small quantity of the main matter (Fig. 2d).

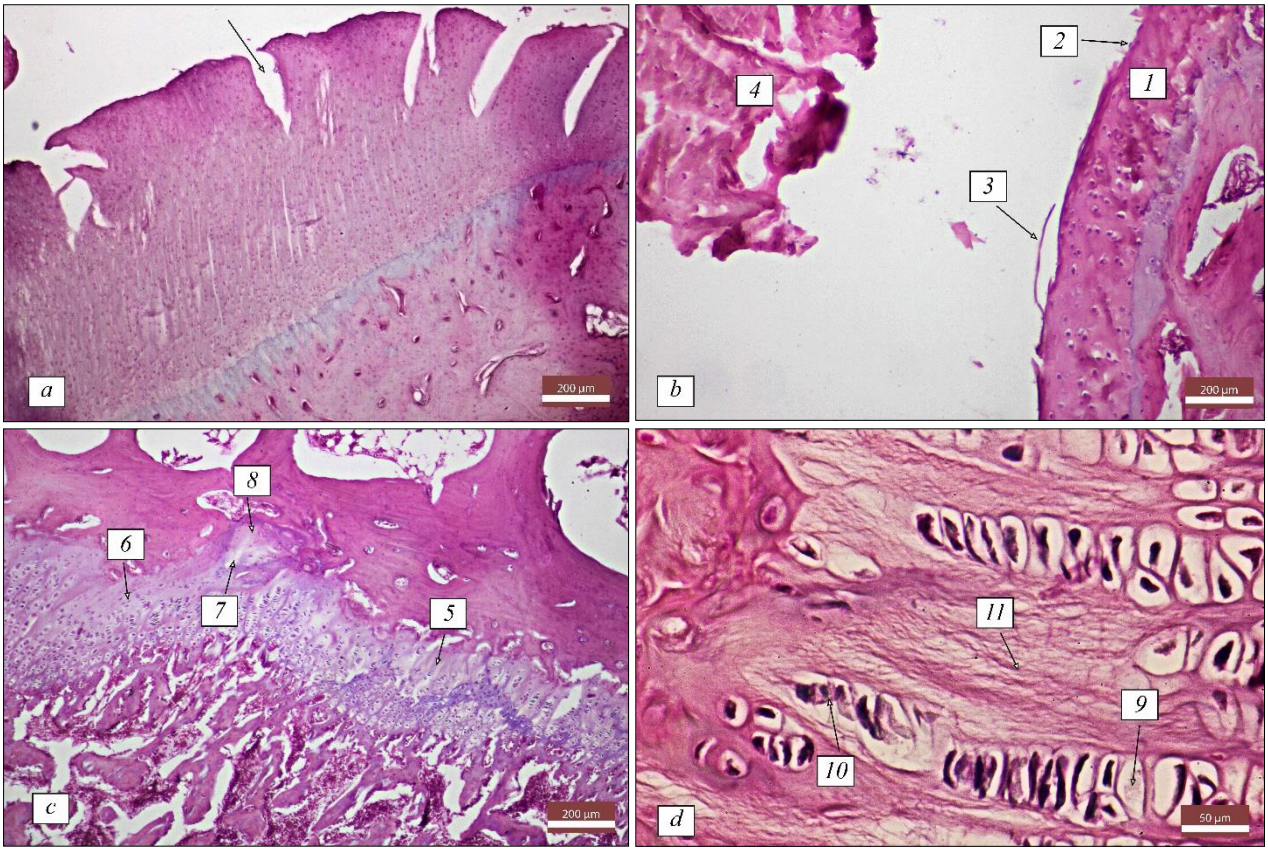

Fig. 2. Structural changes in the tissues of the ankle joint of the rabbit on day 14 after injecting retinyl acetate: *a* – articular cartilage of the femur epiphysis: a superficial fissure of the articular cartilage (indicated with arrow); *b* – joint cartilage of the femur epiphysis; *c* – epiphyseal plate of the femur; *d* – epiphyseal plate of the tibia: *1* – thinned joint cartilage; *2* – absence of perichondrium; *3* – delamination of a region of the perichondrium from the surface of the articular cartilage; *4* – necrosis and destruction of the synovial sheath; *5* – poor staining of the cartilaginous tissue matrix; *6* – random distribution of chondrocytes; *7* – a cavity in the cartilaginous tissue; *8* – a protrusion of the cartilaginous tissue into the spongy tissue between the joint cartilage and epiphyseal plate; *9* – necrosis of a chondrocyte in the region of proliferation of cartilage cells; *10* – degradation of chondrocytes near the spongy bone tissue between the joint cartilage and epihyseal plate; *11* – fibers of the intercellular substance of the cartilaginous tissue

On day 21, the degradation of the cartilage progressed: the fissures reached deep layers, coming into contact with the subchondral bone; proteoglycans were absent in most areas of the cartilage, as confirmed by the absence of staining with Safranin O; decrease in the number of vital chondrocytes; and the presence of necrotic zones.

Other changes included: a significant thickening of the subchondral plate through remodeling; the expansion of the cavities in the subchondral bone; the emergence of areas of osteosclerosis and osteoporosis; the initial formation of osteophytes on the edges of the joint surface; pronounced chronic inflammation, with the expansion of the fibrous tissue; and an increase in the number of blood vessels, indicating active angiogenesis.

During this period of observations, on large areas under the joint cartilage, we found the fibrous connective tissue, not the bone tissue. The same tissue also replaced the red bone marrow in the bone diploe (Fig. 3a, 3b). In our opinion, the expansion of the fibrous connective tissue between the articular cartilage and the spongy bone tissue beneath it was due to two factors. First of all, this tissue replaced the defects that have formed as a result of lysis of the cartilaginous tissue, which was recorded on day 14.

The expansion of the fibrous connective tissue into the diploe of the spongy bone tissue, located between the joint cartilage and the epiphyseal plate, in our opinion, could be due to the cessation of hematopoiesis in this region. Because the fibrous connective tissue replaces various defects in animals, the disappearance of the bone marrow in this area of the bone tissue of epiphysis could provoke the expansion of the fibrous connective tissue in the region of the bone marrow.

Another explanation for why the fibrous connective tissue expanded in the bone tissue diploe beneath the articular cartilage is that

the rabbits' organism may have attempted to strengthen the tissues in this region and thus compensate the decrease in the overall strength of the ankle joint caused by the degeneration of the articular cartilage.

We should particularly note that on the boundary between the connective and cartilaginous tissues, there were numerous chondroclasts – cells that resorb the cartilaginous tissue (Fig. 3a). They were large, polymorphic, multinuclear cells, with lysis of the adjacent cartilaginous tissue observed near their cytoplasm (Fig. 3c, 3d). Microscopic changes in other areas of the epiphyses of the femur and tibia on day 21 after administration of retinyl acetate were similar to those in the previous observation period. In our opinion, this could be associated with the fact that the drug we injected in the joint cavity first of all acted in the region of administration – i.e. toward the joint cartilage.

On day 28 of the study, we observed almost complete destruction of the cartilage in the areas of maximum loading. Furthermore, we saw deep fissures that reached the subchondral bone, the cartilage losing its structure and shock-absorbing properties, and the residual chondrocytes were surrounded by degenerative changes in the matrix (Fig. 4a, 4b).

We observed a pronounced osteosclerosis of the subchondral plate; the expansion of osteophytes, which usually change the configuration of the joint surface; the enlargement of the microcavities; and a partial collapse of individual areas of the bone tissue. On the side of the synovial sheath, we saw total fibrosis of the membrane, which limited its function, persisting chronic inflammation with residual lymphocyte infiltrates, and an increase in the number of vessels, although the capillaries with thin walls prevailed.

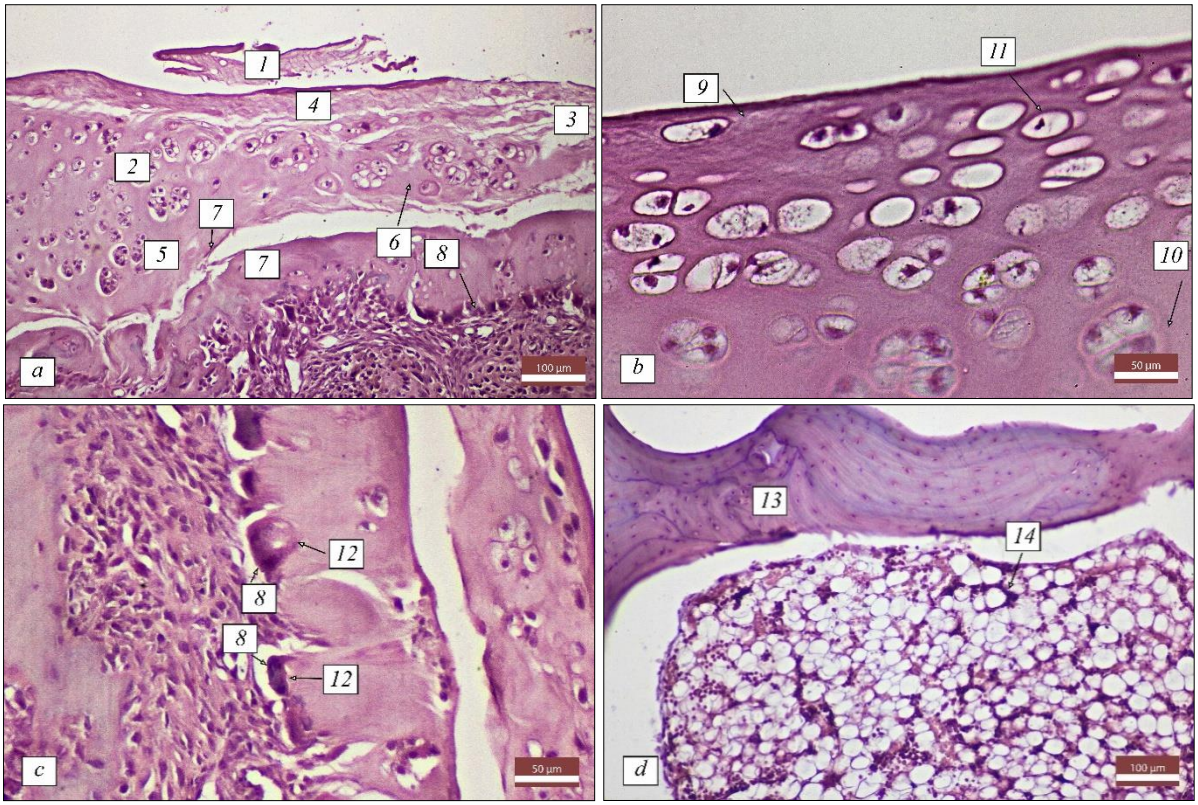

Fig. 3. Structural changes in the tissues of the ankle joint of the rabbit on day 21 after injecting retinyl acetate: *a* – femur epiphysis; *b* – articular cartilage of tibia epiphysis; *c* – articular cartilage of femur epiphysis; *d* – spongy bone between the joint cartilage and epiphyseal plate of the tibia: *1* – detached fragment of the joint cartilage in the joint cavity; *2* – partially separated fragment of the articular cartilage; *3* – destruction of the articular cartilage; *4* – necrosis of the articular cartilage; *5* – isogenic group of chondrocytes; *6* – empty lacunae lacking chondrocytes in an isogenic group; *7* – absence of chondrocytes in the joint cartilage; *8* – chondroclasts; *9* – degradation of chondroblast; *10* – degradation of chondrocyte; *11* – an empty lacuna lacking chondroblast; *12* – lysis of the cartilaginous tissue; *13* – immature bone tissue; *14* – absence of osteoblasts on the surface of bone tissue

On day 35 after injecting retinyl acetate, compared with the previous monitoring period, we observed a much more significant degradation of the articular cartilage, and found cavities of different sizes and forms in its cartilaginous tissue (Fig. 5a).

The microscopic changes in the spongy bone tissue between the articular cartilage and epiphyseal plate were the same as in the previous periods of observations.

In the epiphyseal plate, aside from changes observed in the previous periods, we noted necrosis and the degradation of chondrocytes on the diaphyseal side (Fig. 5b), while registering no hematopoiesis in diploe of the spongy bone tissue on the diaphyseal side (Fig. 5c, 5d).

Thus, injection of retinyl acetate caused significant changes in the structures of the epiphyses of both the femur and tibia. At the same time, these changes were similar to those in the epiphyses of both bones forming the ankle joint, and first of all were characterized by a significant degradation of the articular cartilage, and, moreover, partial necrosis and the destruction of the joint's synovial sheath. However, with time, the pathological process also spread to all the tissues below the epiphyses of both bones. At the same time, the farther it was from the joint cartilage, the later and less extensive the damage occurred.

In the spongy bone tissue located between the joint cartilage and the epiphyseal plate, we observed disorders, with the cessation of both endochondral and intramembranous osteogenesis. At the same time, the red bone marrow was disappearing, with a respective stoppage of hematopoiesis in this region of the bone.

In the epiphyseal plate, we registered a significant impairment of endochondral osteogenesis of the bone tissue located between the joint cartilage and epiphyseal plate. At the same time, malfunctioning osteogenesis toward the diaphyses of the femur and tibia was insignificant. However, at the end of the experiment, we observed the disappearance of the red bone marrow with the resulting stoppage of hematopoiesis in the spongy bone tissue between the epiphyseal plate and diaphysis.

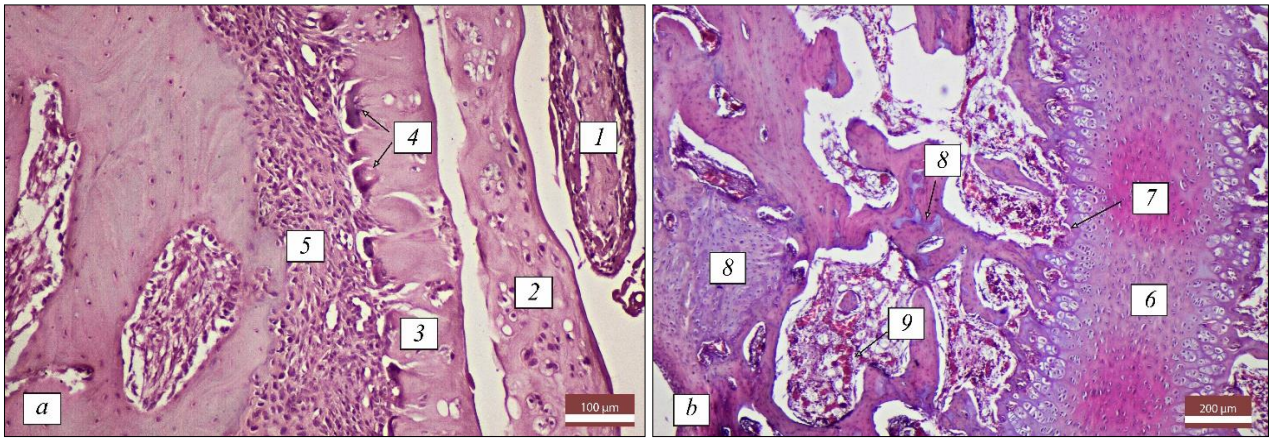

Fig. 4. Structural changes in the tissues of ankle joint of the rabbit on day 28 after injecting retinyl acetate: *a* – tibia epiphysis; *b* – epiphyseal plate of the tibia: *1* – detached fragment of the joint cartilage in the joint cavity; *2* – partially separated fragment of the joint cartilage; *3* – joint cartilage; *4* – chondroclasts; *5* – the fibrous connective tissue; *6* – random and dense arrangement of chondrocytes; *7* – intensive osteogenesis; *8* – fragments of the cartilaginous tissue in the bone tissue of spongy bone between the joint cartilage and epiphyseal plate; *9* – hyperemia of the bone marrow

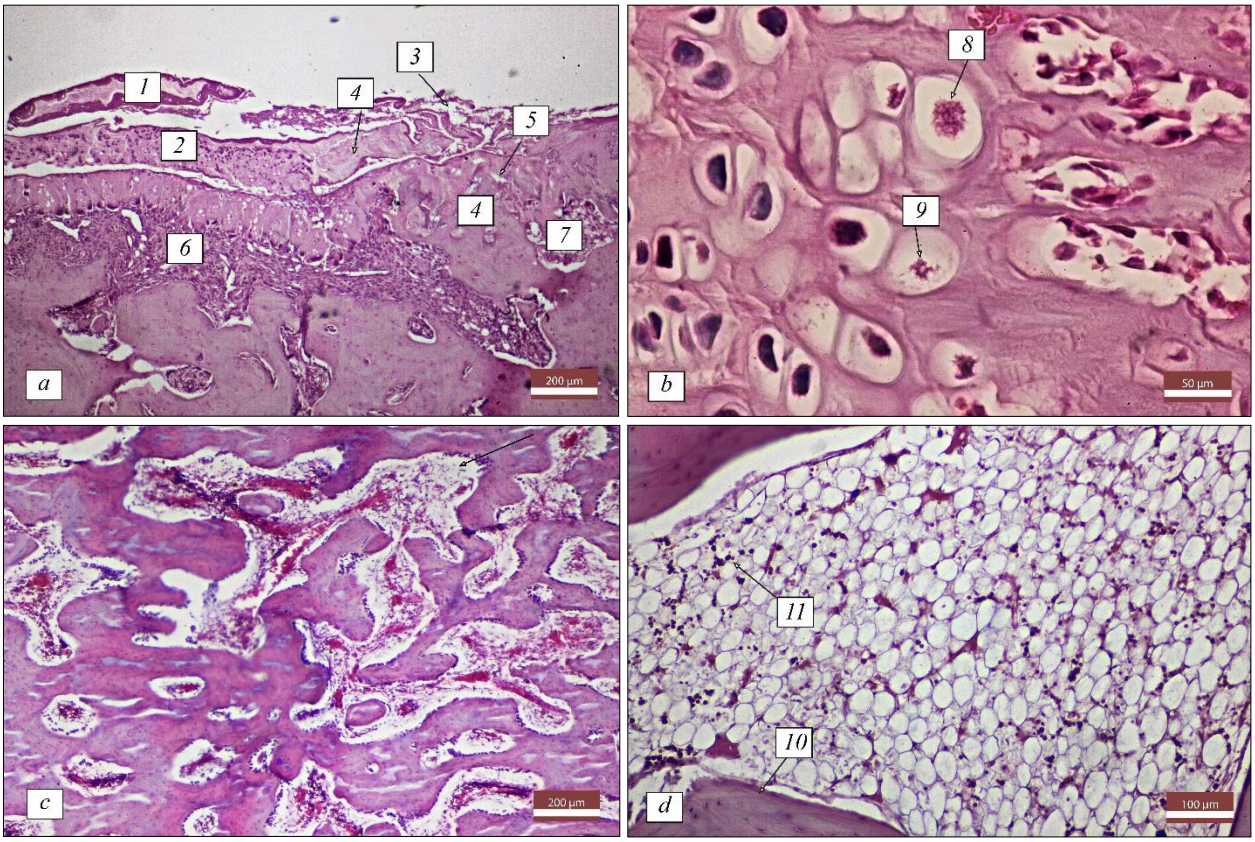

Fig. 5. Structural changes in the tissues of the ankle joint of the rabbit on day 35 after injecting retinyl acetate: *a* – the femur epiphysis; *b* – region of the epiphyseal plate of the tibia on the diaphyseal side; *c* – absence of hematopoietic cells in the diploe (indicated with arrow); *d* – the spongy bone tissue under the joint cartilage: *1* – a detached fragment of the joint cartilage in the joint cavity; *2* – a partially separated fragment of the articular cartilage; *3* – destruction of the articular cartilage; *4* – necrosis of the articular cartilage; *5* – cavities in the articular cartilage; *6* – subchondral fibrous connective tissue; *7* – fibrous connective tissue in the bone diploe; *8* – necrosis of chondrocyte; *9* – degradation of chondrocyte; *10* – the bone tissue; *11* – small accumulation of hematopoietic cells in the bone marrow

Discussion

Creating animal model of gonarthrosis is necessary for studying the pathophysiological changes and developing methods to treat this pathology (Malfait et al., 2015). However, the choice of methods for inducing gonarthrosis in animals is a subject of ongoing debate. In our study, we chose chemically induced gonarthrosis, which requires more time for pathological changes to develop.

To assess the severity of osteoarthritis of both the cartilage and periarticular tissues, all used images contained all structures of the ankle that cover the joint cartilage, subchondral bone, margin of the joint, synovial sheath, capsule of the joint, and surrounding ligaments and muscles (Grote et al., 2022).

The histological changes observed in the ankle joints of the rabbits suffering osteoarthritis indicate a complex pathological process that affects the cartilaginous tissue, subchondral bone, and synovial bone. The main signs of osteoarthritis are progressing destruction of the cartilage, remodeling of the bone, and inflammation. The results of our studies on chemically induced osteoarthritis of the ankle joint align with the results of the studies by Sharifah Zakiah Syed Sulaiman

on surgically and chemically induced osteoarthritis in rabbits (Syed et al., 2022).

In the cartilaginous tissue, changes occurred in the thickness of the articular cartilage. In particular, the cartilaginous tissue progressively thinned due to the loss of chondrocytes and the degradation of the extracellular matrix. Staining with safranin O revealed the decline in color intensity of the area of the articular cartilage, indicating the loss of proteoglycans. The surface area of the joint cartilage was covered with microfissures, which deepened to the middle layers of the cartilage. In the studies on surgically induced models of osteoarthritis in rodents, Ikufumi Takahashi observed the degradation of the cartilage and death of chondrocytes, which is consistent with the results of our studies (Takahashi et al., 2024).

A vitally important role in the support of homeostasis in the joints is played by the subchondral bone. Extensive studies over the past several decades have been focused on understanding how cartilage regeneration in osteoarthritis leads to subchondral remodeling (Dong et al., 2024).

In the area of the subchondral bone, we saw bone remodeling, with the formation of sclerotic regions, and the formation of bone outgrowths on the edges of the joint surface, which is a typical feature of osteoarthritis. Some areas had damages that formed through resorption of the bone tissue. Our results align with other studies (Nagira et al., 2020).

Our results corroborate the findings by Yue et al. (2021). The authors described the damages to the cartilage and the changes that occurred in the tissues under the joint cartilage.

The histological changes in the synovial sheath included the increase in the number of cells, infiltration of the synovial tissue by lymphocytes, macrophages, and plasmatic cells, and the loss of elasticity of the synovial cells in late stages of the disease due to fast deposition of collagen fibers. Our findings are consistent with the studies by Veronesi et al. (2022) on guinea pigs, although the authors argue that histology of non-decalcified tissues remains the best choice for studying the joint tissues, using immunoglobulin chromatography and semi-quantitative histological assessments. In our studies, the assessment of the changes without conducting decalcification was challenging, and therefore we chose to perform decalcification and in-detail histological study of the structures micro- and macroscopically. Our results are similar to those yielded by Zabrzyńska et al. (2024).

Additionally, active angiogenesis was observed in the synovial sheath and subchondral bone, which is a response to the tissue's attempt at regeneration.

Formation of osteophytes and sclerotic changes in the subchondral bone can be considered as compensatory mechanisms for decreasing the loading on the affected areas. The results we obtained are consistent with Davis et al. (2023), although the authors analyzed spontaneous, not induced, osteoarthritis.

Different kinds of induced osteoarthritis were analyzed by Longo (2023). During chemically induced OA, similarly to our studies, the structural changes between days 21 and 35 required more time to become notable, becoming more pronounced on day 35 than on day 28.

Inflammatory processes in the synovial sheath and increase in angiogenesis indicate the participation of the immune system in the osteoarthritis pathogenesis. This highlights the necessity of using anti-inflammatory therapy when treating this disease.

Further studies are required to corroborate these conclusions.

Conclusion

Changes in the structure of ankle joint of rabbits with osteoarthritis reflect the progression of pathological process that affects all tissues of the joint. The degradation of the cartilage, remodeling of the subchondral bone, chronic inflammation of synovial sheath, and damage to the ligamentous apparatus caused functional insufficiencies of the joint, typical for later stages of osteoarthritis.

The histological changes in the tissues of the ankle joint of the rabbits with osteoarthritis demonstrate the progressing character of cartilage degeneration, remodeling of the subchondral bone, and development of chronic inflammation in the synovial sheath. The most pronounced changes were observed on days 21 and 27 after the formation of pathological process, which indicates the transition of osteoarthritis into a chronic stage. These data are important for the understanding of the osteoarthritis pathogenesis and development of therapeutic approaches.

Authors declare no conflict of interests with respect to this paper.

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