ULTRASTRUCTURAL INVESTIGATIONS ON STIFLE JOINT ARTICULAR CARTILAGE IN DOGS TREATED INTRAARTICULARLY WITH SODIUM MONOIODOACETATE

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Abstract. The aim of the study was to investigate the fine ultrastructural changes in the articular cartilage occurring during the development of an experimental canine chemical model of osteoarthritis (OA, DJD) by intraarticular injections of sodium monoiodoacetate (MIA). A process of activation of the cells from the superficial layer was established. The transmission electron microscopy showed significant changes in the surface layers. The lamina splendens began to disrupt, at certain points became thicker and some parts of it were mixed with the underlying matrix. In the territorial matrix of the surface layers, the proteoglycan content was significantly increased. The characteristics of the collagen network changed together with the links between collagen fibres and proteoglycan complexes. In the isogen group of chondrocytes, processes of layer destruction and osteophytic formation took place on the 150th day after injection. A special attention was paid on changes involving proteoglycan complexes within the matrix of articular cartilage in order to establish the alterations resulting in formation of osteoarthritis. In conclusion, the progressive loss of chondrocytes resulted in histologic changes, including collapse of the cartilaginous matrix, fibrillation and osteophyte formation, similar to changes occurring in the naturally acquired OA.

Keywords: Ultrastructure, articular cartilage, osteoarthritis, dog, stifle, sodium monoiodoacetate.

ŠUNŲ KELIO SĄNARINIŲ KREMZLIŲ ULTRASTRUKTŪRINIAI POKYČIAI SULEIDUS Į KELIO SĄNARIO ERTMĘ NATRIO MONOJODO ACETATĄ

Santrauka. Šio darbo tikslas – eksperimentiniu būdu sukelti šunims kelio sąnario osteoartritą ir ištirti ultrastruktūrinius kelio sąnarinių kremzlių pokyčius, kurie atsiranda suleidus natrio monojodo acetatą. Stebint elektroniniu mikroskopu paviršinių sluoksnių ląsteles, pastebėti ryškūs pokyčiai. Sąnario sienelės vidinis sluoksnis suiręs, atskirose vietose suplonėjęs arba susimaišęs su gilesne tarpląsteline kremzline medžiaga. Tuose pačiuose sluoksniuose nustatytas padidėjęs proteoglikanų kiekis. Kolageno skaidulų charakteristika kinta kartu su proteoglikano kompleksais. Praėjus 150 dienų po injekcijos atsiranda pokyčiai ne tik chondrocitų izogeninėse grupėse, bet yra ardomi sąnario sluoksniai, vystosi kaulinės ataugos.

Ypatingas dėmesys darbo metu buvo skirtas proteoglikanų kompleksų ir tarpląstelinės kremzlinės medžiagos tarpusavio ryšiui, jų įtakai osteoartrito formavimuisi. Išvada: histologiniai tyrimai parodė chondrocitų sumažėjimą, tarpląstelinės kremzlės medžiagos suirimą, kremzlinės medžiagos suminkštėjimą ir kaulinių ataugų formavimąsi. Tokie patys pokyčiai vyksta natūraliomis sąlygomis besiformuojant osteoartritui.

Raktažodžiai: ultrastruktūra, kremzlės medžiaga, osteoartritas, šuo, kelio sąnarys, natrio monojodo acetatas.

Introduction. Several factors as biomechanical instability (Mockowitz, 1970, 1973, 1980), reactivity (Muray, 1956), trauma (Vasilev and Vidinov, 1979; Higgs and Young, 1996), climate etc. are reported to be involved in the development of degenerative joint disease (osteoarthritis). In order to evaluate the influence of the specific chemical factors, it is essential to establish the fine mechanisms of osteoarthritis development. Several researches on the articular cartilage claim that one of the basic components of the interterritorial matrix, the aggrecan (protein-polysaccharide complexes) is extremely sensitive to changes in the synovial fluid. This is confirmed by the experiments carried out on animals, used as experimental models of mechanical (Murray 1964; Pond and Nuki, 1973; Marijnissen *et al.*, 2002) and chemical (Gustafson *et al.*, 1992; Bovine *et al.*, 2003) osteoarhritis. However, these experiments put emphasis mainly on the qualitative and not on the quantitative alterations in the articular cartilage.

The aim of the present study was to investigate the ultrastructural changes in the articular cartilage matrix from dogs with experimentally induced osteoarthritis via electron microscopy and ultrastructural techniques, and also to perform a quantitative computer analysis of the changes of concentration and distribution of proteoglycan complexes during experimental osteoarthritis.

Materials and Methods. Eighteen healthy dogs aged 1.5 to 2.5 years from both genders were used as model of an experimental osteoarthritis. The experiment was approved by the Committee on Animal Experimentation at the Trakia University, Stara Zagora, Bulgaria and was performed according to the recommendations of Directive 86/609/EC from November 24, 1986.

The stifle osteoarthritis was induced by injection of sterile 0.9% NaCl aqueous solution of sodium iodoacetate (MIA) (MERCK-Schuchardt, # S05800 228**)** into the joint cavity of the left leg. This chemical inhibits glyceraldehyde-3-phosphate dehydrogenase activity in chondrocytes, resulting in disruption of glycolysis and cell death. The progressive loss of chondrocytes results in

histologic and morphologic changes in the articular cartilage, closely resembling those seen in natural OA in animals and humans (Bovine *et al.*, 2003). We used 10 weekly joint injections of MIA (0.12; 0.14; 0.16; 0.26; 0.36; 0.96; 1.28; 3.00; 5.00 and 10.0 mg/kg) under sterile conditions to induce DJD. The right contralateral joints served for control purposes.

The samples of cartilage for electron microscopy were obtained on the day 150 after the first injection of MIA by shaving thin pieces from the medial femoral condyle of each knee joint in way so that the orientation was retained. They were examined by a HITACHI 604 A electron microscope after routine preparation of specimens. This study was performed for demonstrating proteoglycan complexes of the intercellular cartilage matrix according to the method of Shepard and Mitchel (1965), using Safranin O for this purpose. The received electronogrammes were analysed with image analyser "Olympus"-Version 4.5. For this purpose electronogrammes (Fig. 4) with the same augmentation, made under identical conditions, were calibrated for size, density and intensity of proteoglycan complexes.

Results.

Gross pathological changes

The joint capsule of osteoarthritic joints was thickened and the colour of synovial layer was altered and appeared dark-grey vs control joints and had single petechiae in peripheral zones. The periarticular and subpatellar fat tissue was dark yellow, with single petechiae whereas the contralateral, healthy joints were almost white. The cartilage surfaces of tibia, femur, patella and meniscuses lost their smoothness and shine. Their colour was grey-bluish thus differing from the white to pale rose control joints. In some areas of the articular surface clots of coagulated proteins or other blood elements was found. On areas subjected to the greatest strain (medial femoral condyle, patellar groove), longitudinal linear abrasions and irregularities were observed. The cranial cruciate ligament was dark grey and its cross-section was twice thinner than that of the healthy contralateral joint. Sites with osteophytic formations were visible in the insertion points of colateral ligaments. The joints with OA evidenced a noticeable instability in the anterior-posterior direction during passive movements.

Ultrastructural changes in articular cartilage

The investigations performed 150 days after the last MIA injection showed significant changes in the ultrastructure of the articular cartilage. The transmission electron microscopy of the superficial layer showed significant changes in lamina splendens and in some of the superficial layer cells. Lamina splendens (consisting of proteoglycans of the tangential layer and synovial fluid) began to disrupt and became thicker and well designated by the underlying intercellular matrix and the tangential layer. It consisted of corrugated electrone-dense particles, among which the parts was absent (irregular light regions are seen). Some bundles of collagen fibrils were disarranged and in contact with the intraarticular cavity (Fig.1). The computer analysis of the proteoglycan complexes revealed that the aggrecans consisted of 3 types proteoglycan subunits – small, medium and large ones. The number of the small proteoglycan subunits was high and that of the large proteoglycans – low. The ultrastructural survey for proteoglycans showed changes in their distribution. The proteoglycan content in the territorial matrix of the superficial layer was significantly increased (Fig. 2). The characteristics of the collagen network were changed together with the links between collagen fibres and proteoglycan complexes. The links between the collagen fibres, maintained by the proteoglycan complexes created a web-like appearance of the fibrillar component of the matrix, which was slowly decomposed. The collagen fibres formed a solid bundles. In the isogen group processes of layer destruction and osteophytic formation took place. During the 150-day period after MIA injection, the number of degenerated cells increased and the concentration of proteoglycan complexes in all cartilage layers was altered. Their concentration diminished in the surface and middle layers, both in the territorial and interterritorial matrix (Fig. 3). Chondrocyte loss was complete to the deep zone. Significant proteoglycan loss can be detected all the way to the tide mark. In the deep cartilage layers, proteoglycan concentration decreased in the territorial and increases in the interterritorial matrix. The image analyzer survey showed a total decrease in the matrix proteoglycan content. The distribution and correlation of proteoglycans were also changed. The number of middle and large proteoglycan subunits increased whereas that of the small decreased (Fig. 4).

Discussion. Comparing our results to the normal ultrastructure of the canine articular cartilage (Lust *et al.*, 1972; Horky and Tichy, 2004) it is clear that the articular cartilage matrix was changed under the influence of the chemical agent applied into the joint cavity. The effective dose of monoiodoacetate used by us was about 10 mg/kg. It was considerable higher than that used by Stobie *et al.*, (1994), who administered doses of 0.37-0.50 mg/kg but in fact, did not observe the wanted result. The MIA application resulted in disorganization of the articular cartilage layers. Similarly to previous studies, the changes were shown to affect the proteoglycan complexes of the territorial matrix (Vasilev and Vidinov, 1979; Wolff *et al*., 1995; Papadopoulou, 1999, Bovine *et al.*, 2003). The biochemical analysis of the articular cartilage of osteoarthritic knees shows abrupt or slow alterations in the concentration of different components. It is a common belief that the proteoglycan amount decreases during the osteoarthrotic process (Higg and Young, 1996). However, it is accepted that this reduction is not diffuse, but affects certain parts of the cartilage, where the electron microscopical changes are most significant. In certain areas, those with the greatest loading, the biochemical data correspond to our ultrastructural findings. In other parts, the proteoglycan concentration is normal or close to the normal values. Our research illustrated that the changes firstly affect single cells and their territorial matrix. The interterritorial matrix is involved and distinct layers are destroyed.

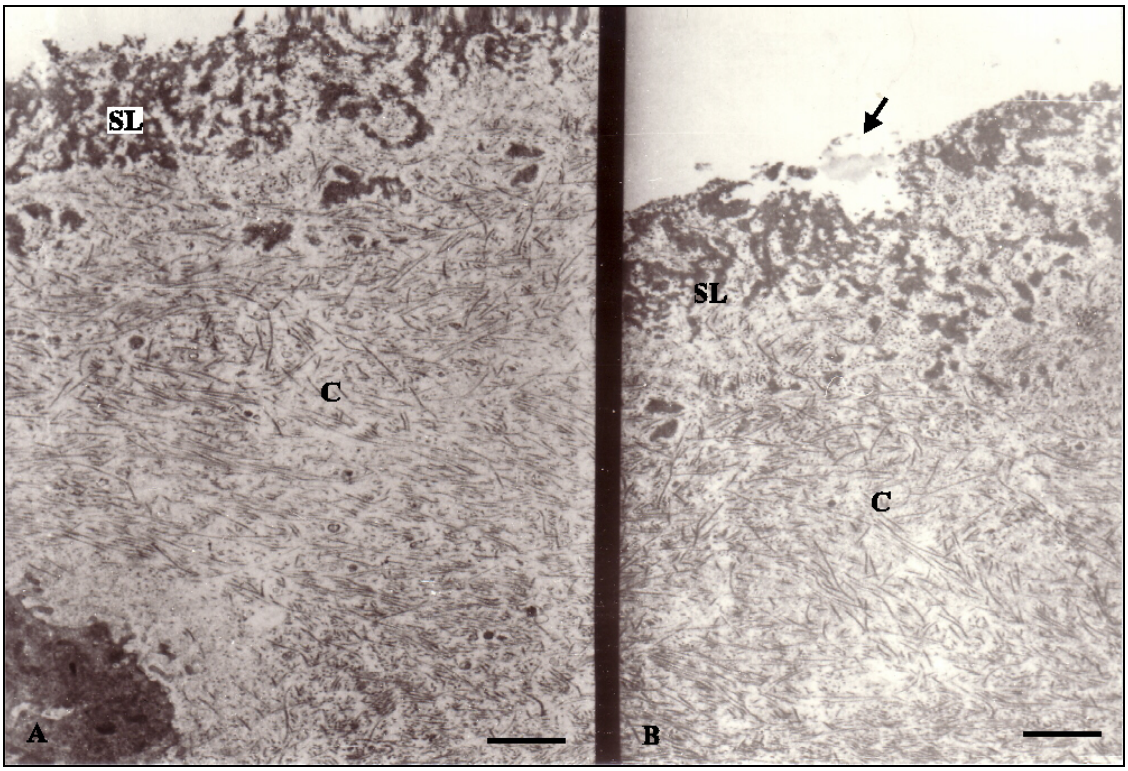

Fig. 1. **Superficial layer of articular cartilage in two dogs (A and B).** Lamina splendens (consisting of electron dense particles, among which irregular light areas are seen) has become thicker and well designated by the underlying intercellular matrix and the tangential layer; bar = 1 μm. SL= superficial layer; C=disarranged bundles of collagen fibrils.

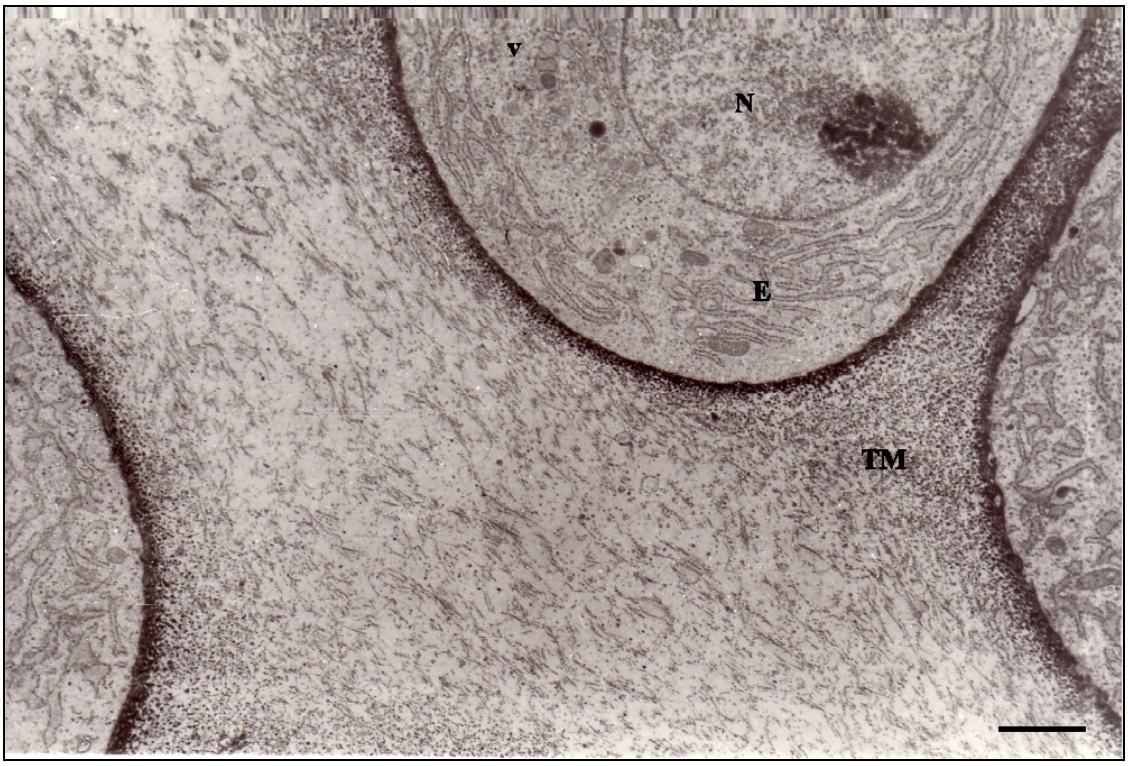

Fig. 2. **The chondroblasts from the superficial layer are activated and the proteoglycan content in the territorial matrix is significantly increased;** bar = 2 μm. N= nucleus; V= vacuoles; E=endoplasmic reticulum; TM= territorial matrix.

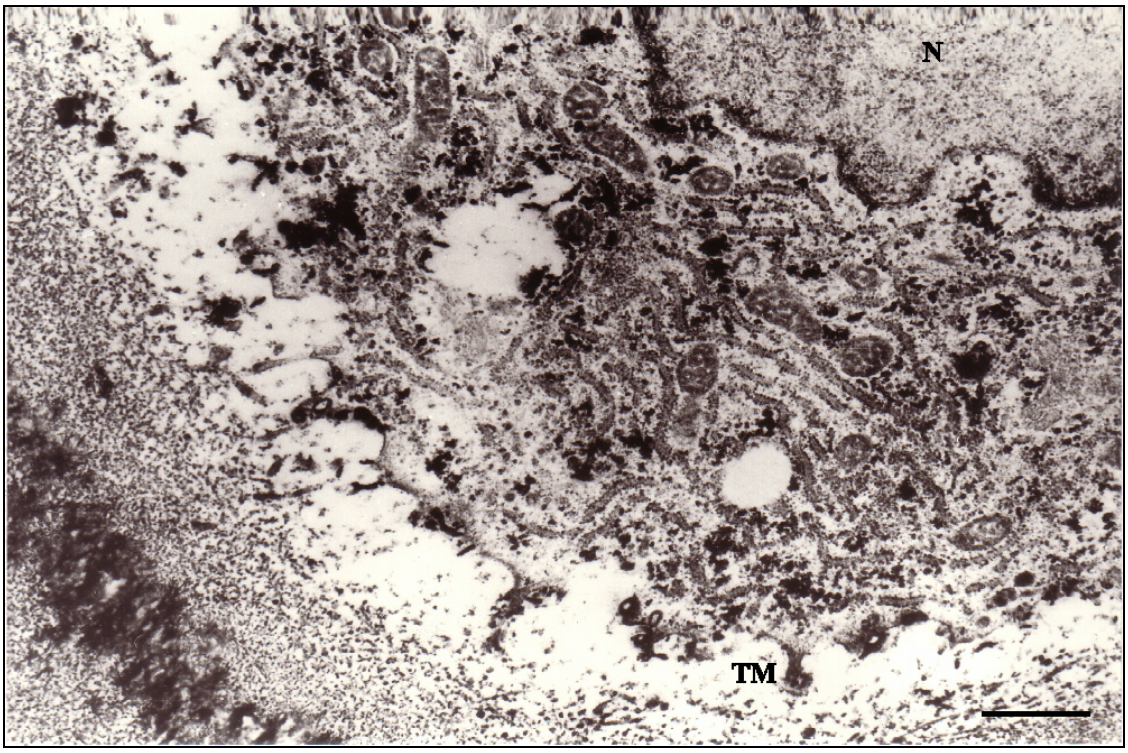

Fig. 3. **The chondroblasts from the deep layer with degenerative changes.** The proteoglycan concentration decreased in the territorial and increases in the interterritorial matrix after the last MIA injection; bar = 2 μm. N= nucleus; E=endoplasmic reticulum; TM= territorial matrix.

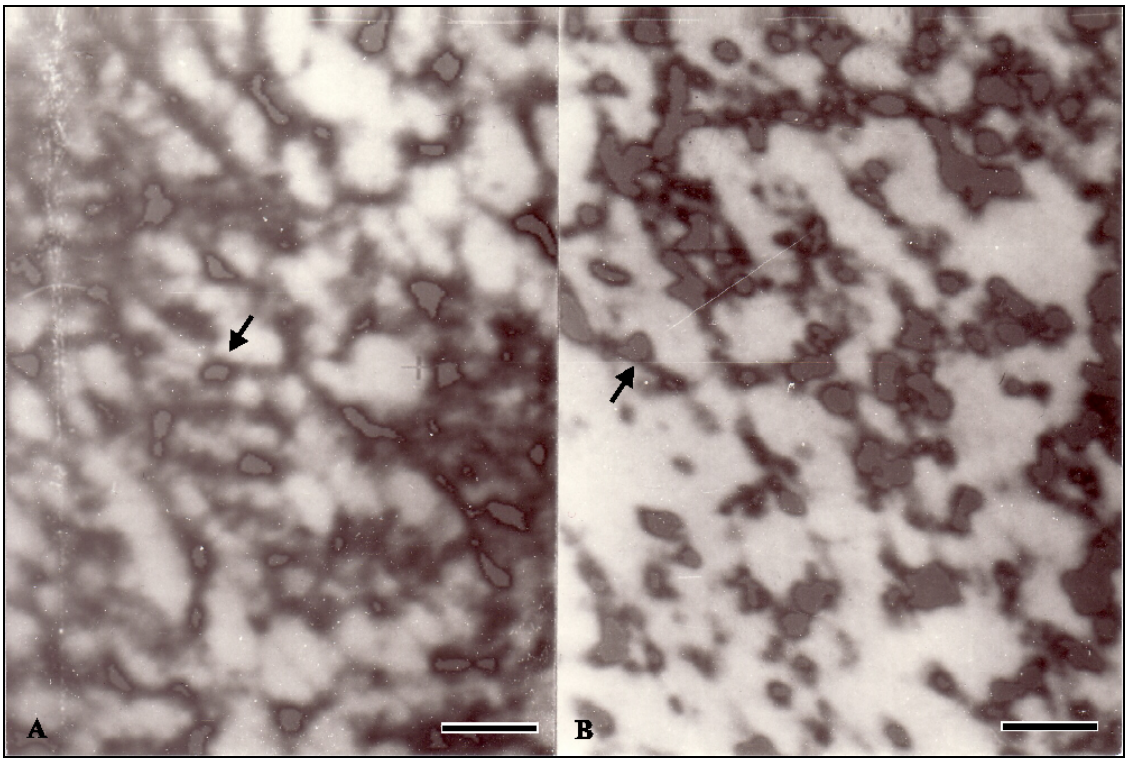

Fig. 4. **Cartilage matrix from an area with severe lesions in knee joint of two dogs (A and B).** The image analyser survey showed that the number of middle and large globular submits (grey spots, arrows) of proteoglycans increases and that of the small ones decreases; bar = 0.5 μm, (Safranin O).

This can be easily seen on the electronogrammes demonstrating the changes in different layers and stages of experimental osteoarthritis (Davies *et al.*, 1962). An activation of the chondroblasts can be seen at the same

time. It is clear in our experiments that the changes involve the structure of globular subunits of agrecan complexes in the territorial matrix. In the final stages of the process the structures of the interterritorial matrix are affected also. The computer analysis of the electronogrammes enables us to detect even the slightest changes leading to osteoarthritis. All discussed changes, cause activation of the surface cells, followed by degenerative changes in them, resulting in the appearance of abnormal components in the intercellular matrix (Vasilev *et al*., 1976). These are the reasons, preventing nutrients, regulating substances such as hormones and cytokines, from reaching the cells through matrix diffusion. In the same time waste products are accumulated in the intercellular space, without the possibility of reaching the synovial fluid and blood circulation (Vassilev and Vidinov, 1976). The same process is observed with the disintegrated matrix macromolecules-waste products of normal or pathologic intramatrix metabolism. Finally, newly-formed abnormal intracellular macromolecules are discharged in the matrix and move freely, before participating in the complicated system of collagen-proteoglycans connections. All this leads to the formation of structures, macroscopically designated as osteophites.

Conclusions. The articular cartilage matrix changes, observed under the influence of an chemical agent (sodium MIA) applied into the stifle joint cavity were similar to those occurring in the naturally acquired OA.

Approximately 150 days after MIA injection, the number of degenerated chondrocyte cells increased and the characteristics of the collagen network changed together with the links between collagen fibres and proteoglycan complexes.

The concentration of proteoglycan complexes (small, medium and large subunits) was altered in all cartilage layers.

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