DOI 10.1007/s10517-021-05365-3

MORPHOLOGY AND PATHOMORPHOLOGY

Morphological Changes in Articular Cartilage and Free-Radical Lipid Peroxidation in Rats with Posttraumatic Osteoarthrosis

R. A. Zubavlenko, S. V. Belova, E. V. Gladkova, O. V. Matveeva, and V. Yu. Ulyanov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 172, No. 8, pp. 248-252, August, 2021
Original article submitted February 5, 2021

Using the rat model of posttraumatic osteoarthrosis of the knee joint induced by surgical transection of their anterior cruciate ligaments, we showed that irreversible loss of hyaluronan by the extracellular matrix of the joint cartilage tissue against the background of oxidative stress accompanied by accumulation of intermediate LPO products in blood serum and formation of thiol system incompetence was one of the key patterns of dystrophic degeneration of the cartilage tissue. Considerable metabolic shifts were associated with structural modification of the articular hyaline cartilage: its thinning and a decrease of chondrocyte density and their abnormal spatial distribution in the matrix with predominance of solitary isolated cells with signs of karyopyknosis and karyolysis.

Key Words: *experimental posttraumatic osteoarthrosis; lipid peroxidation; antioxidant status*

Posttraumatic osteoarthrosis (PTOA) caused by traumatic changes in the joint develops in many active working-age individuals [1], despite complete anatomic recovery of the injured joint tissues provided by surgical treatment in most cases. PTOA is associated with LPO activation and formation of toxic metabolites that produce a destructive effect on the connective tissue structures of the joint [2].

There are various experimental models of osteoarthrosis induced by surgical interventions. The most popular methods are the formation of defects of different area and depth in the loaded articular surfaces as well as surgical lesioning of the joint capsule and ligaments [3].

Research Institute of Traumatology, Orthopedics and Neurosurgery, V. I. Razumovsky Saratov State Medical University, Ministry of the Health of the Russian Federation, Saratov, Russia. ***Address for correspondence:*** 79030230027@yandex.ru. R. A. Zubavlenko

Though the contribution of individual factors to induction of irreversible degenerative and dystrophic changes in the articular hyaline cartilage as the main location of pathological changes in PTOA is studied comprehensively, no unifying concept of the pathogenetic mechanisms leading to the formation a complex of irreversible changes in the affected joint as the organ was proposed. Moreover, the key patterns of phenotypical modification of chondrocytes leading to complete loss of their regenerative potential and cell apoptosis as the major condition for degenerative and dystrophic changes in the cartilage tissue of the affected joints are understudied. Therefore, analysis of the metabolic and structural features of PTOA progress on reproducible experimental models is a promising approach enabling objectification of the leading local and systemic mechanisms of the pathogenesis of joint destruction; it also helps to determine principal directions for creating standardized schemes of pathogenic drug therapy for these disorders.

Here we analyze morphological changes in the articular cartilage and free-radical LPO processes in rats with PTOA of the knee joints.

MATERIAL AND METHODS

The study was performed on 34 outbred albino male rats (age 6-7 months, body weight 290-320 g). All manipulations were performed under anesthesia (Telazol, Xylanit) with strict adherence to Bioethics Principles of Good Laboratory Practice, European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasburg, 1986). The experimental protocol was approved by the Ethics Committee of the V. I. Razumovsky Saratov State Medical University.

In experimental rats (n=14), PTOA of the left knee joint was modeled by transection of the anterior cruciate ligament in the intercondylar area with lesioning of the cartilage and subchondral bone with a lancet tip (a wedge-shaped defect was created on the femoral articular cartilage); after that, the wound was sutured layer-by-layer [5]. In the negative control group (n=8), the joint capsule was opened, but the articular structures were not lesioned; then, the surgical wound was sutured layer-by-layer. The control group comprised 12 healthy animals. All animals were housed in individual cages under standard vivarium conditions at 19±1.7°C and natural light and had free access to water and food.

The general health status of the animals as well as local changes in their knee joints were evaluated weekly throughout the experiment. The circumference of the experimental and contralateral knee joints and skin temperature were measured. On day 28 after PTOA modeling, the blood was collected from the heart into tubes with clot activator (SiO_2) and barrier gel. The animals were euthanized by narcosis overdose; the knee joint tissues were isolated en bloc and after standard histologic processing were stained with hematoxylin and eosin. Morphometric analysis included measurement of the articular hyaline cartilage thickness and area of the nucleus and chondrocyte cytoplasm. Types I and II chondrocytes were differentiated by their nucleocytoplasmic ratio. Type I chondrocytes were located in the surface layer of the articular cartilage and had higher nucleocytoplasmic ratio, while in type II chondrocytes, the nucleocytoplasmic ratio was low (cytoplasm predominated) and they were located deep in the articular cartilage closer to the subchondral plate.

LPO intensity was assessed by accumulation of lipid hydroperoxides in animal serum using Lipid Hydroperoxide (LPO) Assay Kit (Cayman Chemical); activity of the antioxidant system was evaluated by the indicators of total antioxidant status (ImAnOx (TAS/TAC) Kit, Immundiagnostic AG) and thiol status (Thiol-status, Immundiagnostic AG). The level of metabolic processes in the articular hyaline cartilage was assessed by serum hyaluronan concentrations (Quantikine ELISA Hyaluronan Immunoassay; R&D Systems, Inc). All these measurements were performed by ELISA using EPOCH (BioTek) photometer.

Statistical processing of the obtained results was carried out using Statistica 10 software (StatSoft, Inc.). Normality of master sample distribution was checked using the Kolmogorov—Smirnov and Shapiro—Wilk tests. As most of the quantitative variables did not fit the normal distribution law, the nonparametric two-sided Mann—Whitney U test was applied. The quantitative characteristics are presented as Me (25%; 75%). The values were considered significant at $p<0.05$ for all analytical methods.

RESULTS

In 28 days after PTOA modeling, local temperature near the knee increased by 0.4°C in experimental rats in comparison with the initial parameter and the mobility in their experimental joints was limited. In the negative control group, no changes in knee joints were observed.

In rats with PTOA, we observed activation of LPO processes: accumulation of lipid hydroperoxides and intermediate molecular products. One of the key biological effects of these products is destabilization of cell membranes [4,8].

Evaluation of the antioxidant defense system revealed no significant differences between the analyzed groups. However, a significant decrease in activity thiol-containing compounds, both free and protein-bound SH groups [6], was observed in rats with PTOA (Table 1), which can reflect the initial signs of insufficiency of the redox buffer cell system under conditions of excessive utilization of antioxidants aimed at the maintenance of the reducing system.

No significant differences by the studied parameters were revealed between the control and negative control group.

Excessive prooxidant activity resulting from intensified ROS generation and accompanied by failure of individual links of the antioxidant defense system in animals with PTOA was associated with degenerative changes in the extracellular matrix, which was confirmed by an increase in serum hyaluronan (Table 1) and probably resulted from its depolymerization amid poor metabolic environment [2,7].

Histomorphometric evaluation revealed the loss of the articular hyaline cartilage thickness (by 19.5%) in the projection of most loaded areas of the femur

TABLE 1. Serum Concentrations of Markers of LPO and Articular Cartilage Metabolism (Me (25%; 75%)

Parameter	Control group	Experimental group	Negative control group
Lipid hydroperoxides, nM	6.3 (5.7; 7.3) *n*=10	7.6 (6.9; 9.7) *p*=0.03 *n*=14	6.9 (6.5; 7.3) *n*=8
Thiol status, μmol/liter	108.3 (102.3; 117.4) *n*=12	90.8 (78.3; 105.8) *p*=0.02 *n*=14	100.2 (94.0; 118.7) *n*=8
ImAnOx (TAS/TAC), μmol/liter	302.3 (280.1; 317.1) *n*=11	285.4 (272.5; 311.8) *n*=14	297.9 (283.2; 316.2) *n*=8
Hyaluronan, ng/ml	62.6 (58.2; 66.2) *n*=10	102.6 (92.2; 114.9) *p*=0.000025 *n*=16	96.6 (85.6; 105.7) *p*=0.0004 *n*=8

Note. Significance of differences from the control is shown. *n*: number of animals.

and tibia in experimental rats. Moreover, the boundary between the spongy bone tissue and superficial cartilage (Fig. 1, *b*) was blurred in comparison with that in the control group, where the spongy bone tissue was separated from the superficial cartilage with the subchondral plate (Fig. 1, *a*). Phenotypical modification of chondrocytes with signs of karyopyknosis and sometimes karyolysis is worthy of note (Fig. 2,

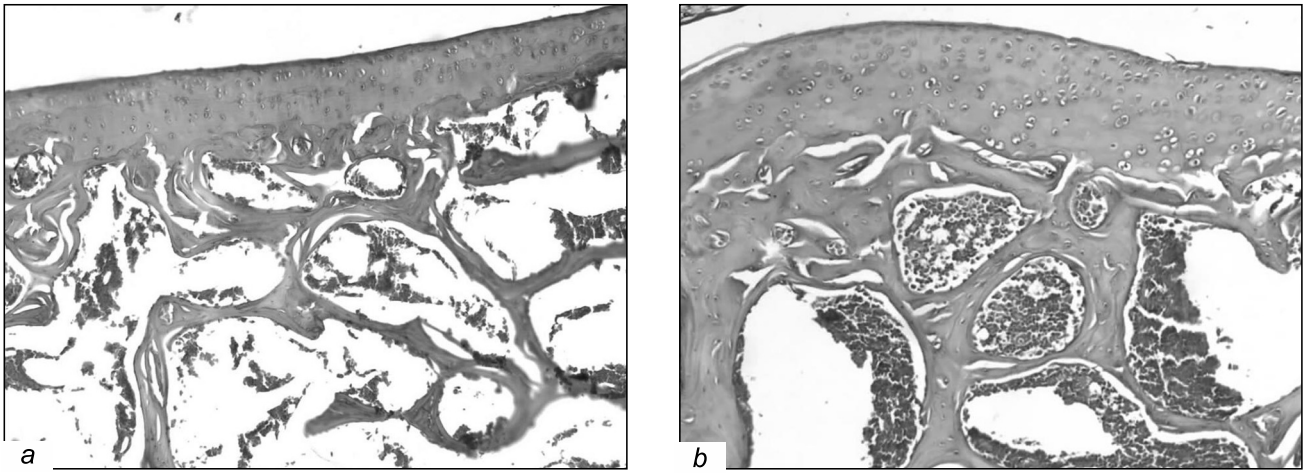

Fig. 1. Fragment of the knee joint from a healthy animal (*a*) and a rat with experimental PTOA (*b*). Hematoxylin and eosin staining, ×10.

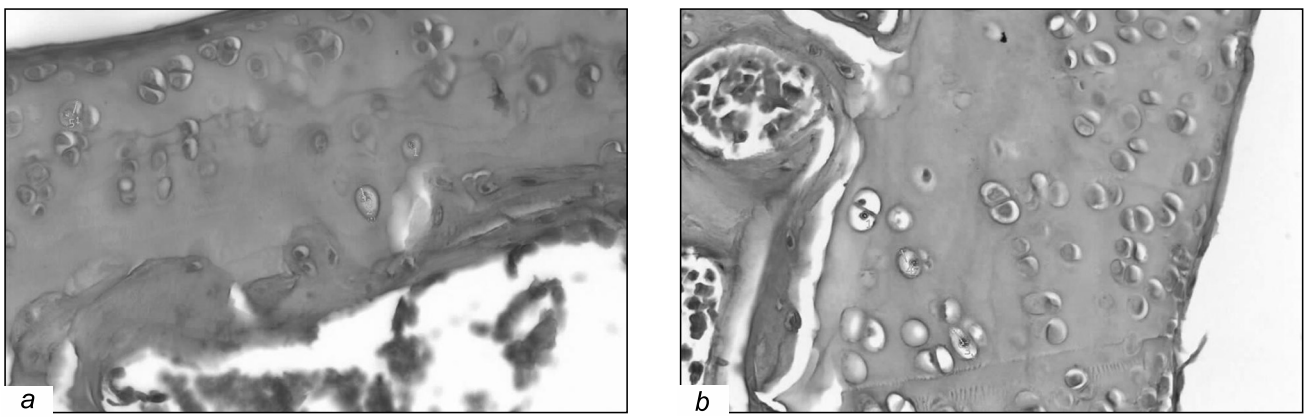

Fig. 2. Superficial cartilage fragment of the knee joint from a healthy animal (*a*) and a rat with experimental PTOA (*b*). Hematoxylin and eosin staining, ×40.

TABLE 2. Results of Histomorphometric Evaluation of Joint Cartilage (Me (25%; 75%); *n*=6)

Group	Nucleus		Cytoplasm	
	type I chondrocytes	type II chondrocytes	type I chondrocytes	type II chondrocytes
Control	0.0057 (0.005; 0.006)	0.0045 (0.004; 0.005)	0.014 (0.012; 0.016)	0.011 (0.010; 0.012)
Experimental	0.0025 (0.002; 0.003) **p*=0.004	0.073 (0.06; 0.09) **p*=0.004	0.0145 (0.013; 0.016) **p*=0.003	0.013 (0.011; 0.014) **p*=0.05
Negative control	0.0053 (0.005; 0.006) **p*=0.003 ^{+}p=0.004	0.0042 (0.004; 0.005) **p*=0.005 ^{+}p=0.004	0.0138 (0.013; 0.015)	0.0123 (0.010; 0.013)

Note. Significance of differences from *control and $^{+}$experimental groups is shown. *n*: number of animals.

Table 2); these changes were most pronounced in type II chondrocytes.

The examination of histological specimens of the hyaline cartilage fragments of the knee joints from animals with PTOA showed abnormal spatial orientation of type I chondrocytes that were mostly isolated (Fig. 2, *b*) in comparison with the control (Fig. 2, *a*). It should be noted that in PTOA animals, type II chondrocytes predominate; karyopyknosis with nucleus shrinkage (by 2 times in comparison with the control) and sometimes karyolysis were found (Fig. 2, *b*).

In rats of healthy control and negative control groups, the thickness of the articular cartilage was <0.15±0.03 mm. The cell elements were presented by round type I chondrocytes that had round nuclei with dense chromatin; these cells formed isogenic groups of 2-4 chondrocytes. Type II chondrocytes formed groups or lay separately (Fig. 2, *a*).

Thus, PTOA in rats resulting from damage to the anterior cruciate ligaments is characterized by activation of catabolic process in the extracellular matrix of the articular cartilage and loss of the key structural component hyaluronan against the background of pronounced prooxidant activity, accumulation of lipid hydroperoxides in systemic blood flow, and the formation of thiol system insufficiency; while the parameters of the total antioxidant activity did not differ from normal. The decrease in the total number of cell elements that mostly underwent phenotypical modification suggesting inhibition of synthetic activity attested to a decrease in the regenerative potential of cartilage tissue.

REFERENCES

1. Zakhvatov AN, Belyaev AN, Atkina NA. Correction processes of free radical oxidation and metabolism of collagen of articular cartilage in experimental knee joint injury. Kafedra Travmatol. Ortoped. 2016;(3):45-49. Russian.
2. Zakhvatov AN, Tarasova TV, Avanesov AM, Zakharkin IA, Khaydar DA, Chekmaeva AA, Sermina TA. Correction of metabolic disorders of collagen in arthritis of posttraumatic genesis. Zdorov'e Obrazovanie XXI. 2018;20(12):193-197. Russian.
3. Kotelnikov GP, Lartsev YuV, Makhova AN. Comparative evaluation of structural changes of joint tissues in various models of experimental arthrosis. Kazan. Med. Zh. 2006;87(1):31-35. Russian.
4. Martusevich AA, Peretyagin SP, Martusevich AK. Molecular and cell mechanisms of singlet oxygen effect on biosystems. Sovremen. Tekhnol. Med. 2012;(2):128-134. Russian.
5. Slesarenko NA, Shirokova EO. Reparative osseo- and chondrogenez in the conditions of induced osteoarthrosis in laboratory animals. Ross. Vet. Zh. Melkie Domash. Dikie Zhivotnye. 2012;(1):6-8. Russian.
6. Altay MA, Ertürk C, Bilge A, Yaptı M, Levent A, Aksoy N. Evaluation of prolidase activity and oxidative status in patients with knee osteoarthritis: relationships with radiographic severity and clinical parameters. Rheumatol. Int. 2015;35(10):1725-1731. doi: 10.1007/s00296-015-3290-5
7. Shimura Y, Kurosawa H, Kaneko H, Nojiri H, Iwase Y, Kaneko K, Ishijima M. Serum hyaluronan levels are associated with disability for activity of daily living in patients with knee osteoarthritis regardless of the radiographic severity of the disease. Osteoarthritis Cartilage. 2018;26(1):S354-S355. doi: 10.1016/j.joca.2018.02.702
8. Yusupov M, Van der Paal J, Neyts EC, Bogaerts A. Synergistic effect of electric field and lipid oxidation on the permeability of cell membranes. Biochim. Biophys. Acta Gen. Subj. 2017;1861(4):839-847. doi: 10.1016/j.bbagen.2017.01.030