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Role of Imbalance of Lipid Peroxidation and Articular Cartilage Remodeling in the Pathogenesis of Early Primary and Post-Traumatic Gonarthrosis in Rats

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We studied serum indicators of LPO and antioxidant system mechanisms in nonlinear male white rats at the early stages of simulated primary knee osteoarthrosis (n=60) and post-traumatic knee osteoarthrosis (n=60) as well as in intact animals (n=20). An imbalance in the peroxidation—antioxidant system with accumulation of lipoperoxides and malondialdehyde along with a decrease in catalase and superoxide dismutase activities was revealed. Early stages of osteoarthrosis were characterized by increased production of primary and intermediate LPO products and exhaustion of the antioxidant system enzymes.

Key Words: *articular cartilage; experimental osteoarthrosis; lipid peroxidation products; activities of antioxidant defense enzymes*

Osteoarthritis or osteoarthrosis (OA) is a widespread joint disease. The development and progression of OA were addressed in many academic and research works, however, the common idea about these aspects has not been eventually formulated [1]. One of the significant pathogenic mechanisms substantially contributing to the formation of permanent structural and metabolic damage to articular cartilage and adhering osseous structures in OA is the progress of profound oxidative stress. The excessive ROS production along with the incompetence of antioxidant defense (AOD) mechanisms results in toxic damage to lipid biolayer of cell membranes in supporting connective tissues [5]. LPO in OA can be triggered by immediate stimulation of cell enzyme systems that contain cyclooxygenase or lipoxygenase as their active components, or by the free-radical mechanism with the formation of ROS [11]. Uncompensated augmentation of prooxidant activity is accompanied by inflammatory destruction of extracellular matrix in the articular cartilage with involvement of proteoclastic systems against the background of significant cytokine imbalance and expression of IL-1β and other proinflammatory mediators. Progressing deficiency of AOD enzymes contributes to the imbalance in the peroxide homeostasis system with predominance of excessive generation and accumulation of primary and final LPO products in biological media [10].

Resident chondrocytes are known to play a key role in the maintenance of adequate homeostasis. These chondrocytes are facultative macrophages essentially involved in the regulation of most catabolic and anabolic processes assisted by matrix metalloproteinases and tissue inhibitors of metalloproteinases as well as ADAMTS proteins including those containing thrombospondin and disintegrin motifs. The cascade of pathological events leading to inflammatory and degenerative modifications of the cartilage tissue in OA is also associated with disruption of chondrocyte cell cycles, their phenotypic modulation, enhanced proliferation, and uncontrolled apoptosis, in particular due to destabilization of cell membranes at all levels [12].

Thus, the role of oxidative stress in the formation of molecular and biochemical patterns of tissue inflammatory degeneration progressing in OA is undeniable.

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However, the mechanisms of early homeostatic disturbances in the articular cartilage are understudied, and the relevance of objectification of certain pathology patterns in experimental simulations is predetermined.

This research was aimed at investigation of the role of LPO and articular cartilage remodeling in the pathogenesis of early signs of primary and post-traumatic OA in rats.

MATERIAL AND METHODS

The experiments were carried out on 18-month-old male white laboratory rats (n=140) weighing 250-290 g. The study was performed in accordance with the principles of Good Laboratory Practice and approved by the Ethics Board of the V. I. Razumovsky Saratov State Medical University (Protocol No. 2, October 2, 2018).

The intact animals (n=20) served as the control. In rats of experimental groups 1-3 (n=20 in each group), primary gonarthrosis (PGA) was simulated in their right (experimental) knee joint through injection of 2 mg dexamethasone followed in 24 h by injection of 10% water suspension of sterile talcum as an abrasive agent [7]. In animals of group 4-6, post-traumatic gonarthrosis (PTGA) was simulated through surgical transection of the anterior cruciate ligaments [4]. The biomechanical destabilization of the joint was verified by the Lachman test that showed the anterior drawer effect in animals lying on their backs with their hind legs bent. This test was aimed at checking the mode of movement in the knee joints at 60° and 90° in no less than three positions of the lower legs: external and internal rotations, and neutral position. The experimental animals were kept in individual cages with 12-h daylight at 19-22°C and 60% humidity with free access to water and standard food. The animals were weakly weighed, the status localis was evaluated by measuring of the circumference of the experimental and control knee joints and the maximum amplitude of extension, and the skin temperature was measured. The observation after OA simulation was performed for 7 days (groups 1 and 4), 14 days (groups 2 and 5), and 21 days (groups 3 and 6).

The blood for biochemical testing was taken from the heart into Vacuette containers with an activator (SiO_2) and separator gel. The serum was obtained by 15-min centrifugation at 2000 rpm. Then the samples were frozen and kept at -80°C. The whole blood was collected with vacuum tubes containing K3 EDTA. Lipid hydroperoxides in rat serum were assayed by ELISA using Lipid Hydroperoxide (LPO) Assay Kit (Cayman Chemical Co.) on an Anthos 2020 microplate spectrophotometer (Biochrom Ltd.). Malondialdehyde (MDA) content in the whole blood was measured in the reaction with TBA by spectrophotometry at 535 nm on a PE-5400 UV spectrophotometer [3]. Catalase activity was measured using a commercial Catalase Assay Kit (Cayman Chemical Co.) according to the manufacture's recommendations in the presence of methanol and hydrogen peroxide by the production of formaldehyde that forms stained complexes with 4-amino-3-hydrazine-5-mercapto-1,2,4-triazole. The degree of total activity of three superoxide dismutase (SOD) subtypes (Cu/Zn, Mn, and FeSOD) was investigated as a catalase synergist using Superoxide Dismutase Assay Kit (Cayman Chemical Co.). SOD activity can also be measured by ELISA using an Epoch microplate spectrophotometer (BioTek) through dismutation with tetrazolium salts and detection of superoxide radicals generated by xanthinoxidase and hypoxanthine.

All manipulations on animals were conducted under combined intramuscular anesthesia of 0.1 ml/kg Telazol (Zoetis Inc.) and 1 mg/kg Xylanit (Nita-Farm); the animals were sacrificed by narcosis overdose.

After sacrifice, the knee joint tissues were removed *en bloc*, put into neutral formalin, decalcified, and processed in ethanol of increased concentration. The paraffin blocks were then cut into 4-7-mm specimens and stained with hematoxylin, eosin, and alcian blue (pH 1.0; pH 2.5). Color and monochrome digital images of articular cartilage specimens were taken with incident light microvisor (Lomophotonica), and evaluated in Microsoft PowerPoint greyscale view mode with points of interest morphometry expressed in pixels. The results were concluded as a mean value acquired in no less than 20 measurements in each of the analyzed topography areas of the load-bearing section in articular cartilage in the projection of tibial and femur median condyles as compared to their unloaded sections and presented percentagewise. All measuring was done as described previously [6].

The findings were processed in Statistica 10.0 (StatSoft, Inc.). The results are presented as Me (Q1; Q3). The differences between the groups were analyzed using the Mann—Whitney U test. The correlations were assessed using the Spearman's correlation test (r) test at $p<0.05$.

RESULTS

A certain level of oxidative stress is known to provide cell growth, proliferation, differentiation, and apoptosis in supporting connective tissues in the physiology norm. In OA the excessive production of hydroxyl radicals by chondrocytes is accompanied by the maintenance of the high level of inflammatory responses underlying degenerative changes in acicular cartilage and phenotypic transformation of cell elements possibly leading to a significant decrease in regenerative tissue potential [6].

A significant increase in serum content of primary LPO products in animals of group 1 (PGA) and group 4 (PTGA) was detected as early as 7 days after simulation of these pathologies (Table 1). It is worth of note that we also registered an increase in secondary LPO products in rats of group 4 evidenced by the rise of blood MDA in comparison with that in group 1 animals and controls. It cannot be excluded that this evidence of excessive prooxidant activity could result from maladaptive reactions in the systemic effect of stress factors (administration of hormonal corticosteroid in PTGA formation and surgery stress in PTGA simulation). This suggestion was implicitly proven by the changes in the activity of enzyme link in the AOD system that showed the compensative catalase increase in animals of the group 1 as well as catalase and SOD increases in group 4. Therefore, OA simulation resulted in the evident metabolic imbalance in the LPO/AOD system promoting the augmentation of irreversible damage to cell membranes. Follow-up observation revealed a gradual decrease in catalase activity by 5% in group 3 (PGA) and by 29% in group 6 (PTGA) possibly suggesting aggravation of the imbalance of mechanisms of biological inactivation of ROS more relevant in the groups of animals with PTGA. There is an opinion that the signs of catalase and SOD activity synergism are observed in the molecular transformation of ROS [5]. This trend was also shown in our research by early signs of experimental knee OA of primary and post-traumatic genesis in rats. Herewith, it should be noted that the serum activity of SOD presented more significant activity dynamics than that of catalase. The revealed functional features of AOD enzyme link in early signs of simulated PGA and PTGA implicitly indicated the significant intensification of intracellular and extracellular dismutation responses; their key biological function was the inactivation of superoxide to form oxygen and hydrogen peroxide [9]. The decrease of intracellular antioxidant potential was also featured by the decline of catalase activity revealed in rats of groups 4, 5, and 6; this potential was associated with the realization of redox processes aimed at the further transformation of hydrogen peroxide, and low molecular weight alcohols and nitrite oxidation.

The correlation analysis revealed no significant relations between the analyzed parameters in animals of groups 1 and 4 as well as in the controls. A moderate correlation (r=0.55, p<0.05) between the generation of primary and intermediate LPO products was found in group 2 rats as well as group 3 rats (r=0.57, p<0.05) in 14 days after PGA simulation. Similar pattern of changes in LPO process was observed in animals with PTGA simulations in 14 days after surgeries and later. By day 21 mild correlation between MDA and LPO was revealed in the animals of group 5 (r=0.55, p<0.05) and group 6 (r=0.57, p<0.05). Therefore, the rearrangement of correlations in the LPO—AOD system along with enhanced generation of primary and intermediate LPO products, and the decrease in the activity of AOD enzymes took place in animals by day 14 of PGA and PTGA simulations.

Normally the avascular and denervated tissue of articular hyaline cartilage is capable of enduring significant axial load; this capacity is mainly ensured by the optimal quantitative ratio and spatial orientation of high molecular weight components within its matrix, glycosaminoglycans, proteoglycans, and collagen in particular [2]. It can be hypothesized that at the initial manifestations of inflammatory destruction, minor loss of glycosaminoglycans can be compensated by an increase in chondrocyte functional activity; this had been indirectly proven in the study of our foreign colleagues [10]. However, phenotypic changes of

TABLE 1. Dynamics of the Content of LPO Products and Activity of Antioxidant Enzymes in Body Fluids of Rats with Simulated PGA and PTGA (Me (Q1; Q3)

Group	LPO, nmol/ml	MDA, nmol/ml	Catalase, nmol/min/ml	SOD, U/ml
Controls (intact rats)	4.75 (4.5; 4.9)	2.33 (2.15; 3.45)	15.84 (14.88; 17.06)	40.74 (40.16; 41.73)
PGA simulations				
Group 1 (day 7)	5.4 (5.15; 5.95)*	3 (2.70; 3.63)	17.2 (15.99; 18.12)*	41.27 (40.04; 41.90)
Group 2 (day 14)	6.8 (6.6; 7.1)*+	3.67 (2.83; 4.06)*+	15.95 (15.76; 16.17)+	37.87 (37.31; 38.52)*+
Group 3 (day 21)	7.5 (7.4; 7.5)*x+	3.98 (3.80; 4.11)*x+	15.19 (15.02; 15.28)x+	36.49 (36.17; 36.73)*x+
PTGA simulations				
Group 4 (day 7)	8.25 (8.1; 8.55)*+#	6.67 (6.19; 6.96)*+#	14.68 (13.91; 15.24)*+	37 (36.52; 37.76)*+
Group 5 (day 14)	6.05 (5.81; 6.30)*xo	3.91 (3.62; 4.10)*x	11.605 (11.46; 12.43)*xo	28.71 (27.92; 29.48)*xo
Group 6 (day 21)	6.25 (5.92; 6.51)*#o&	4.21 (3.94; 4.44)*	11.25 (10.81; 11.86)*#o&	29.46 (29.20; 29.94)*#o&

Note. p<0.05 in comparison with *the controls, +group 1, xgroup 2, #group 3, ogroup 4, &group 5.

cells in supporting tissues under unfavorable metabolic conditions can result in the synthesis of incomplete structural components that do not correspond to conformational demands of the microenvironment and increase the antigen load [8].

It is worth of note that the significant irregularities in peroxidation-antioxidant homeostasis in the early stages of simulated knee OA in rats that we revealed were accompanied by changes in proportions of structural macromolecules within the articular cartilage extracellular matrix in the loaded sections of knee joints. For example, in groups 3 and 6 we observed significant ($p<0.05$) decrease by 14.2 (10.1; 15.3) and by 11.7 (9.4; 14.9)% in the content of high-sulfated glycosaminoglycans in articular hyaline matrix.

Therefore, the early signs of simulated PGA and PTGA in rats are accompanied by prooxidant activity along with the incompetence of the enzyme link in the serum AOD system, and the formation of evident correlations between the output of primary and secondary LPO products. The peroxidation—antioxidant imbalance is an early pattern of degenerative and dystrophic changes in articular cartilage with the predominance of catabolic processes leading to permanent loss of high-sulfated glycosaminoglycans in its extracellular matrix.

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