IJDIAS International Journal of Discoveries and Innovations in Applied Sciences

| e-ISSN: 2792-3983 | www.openaccessjournals.eu | Volume: 2 Issue: 4

An Experimental Study of the Use of Intraarticular Platelet-Rich Autoplasma Therapy in Rats with Knee Osteoarthritis

Ziyadullaev Abdusalom Habibullaevich
Teacher of Bukhara State Medical Institute, Uzbekistan

Abstract:

We evaluated the morphological changes in the knee hyaline cartilage in 30 Wistar mature rats with experimental knee osteo- arthritis after intraarticular platelet-rich autoplasma therapy. Knee osteoarthritis has been shown to involve damage and destruction of the articular cartilage associated with vascular proliferation and granulomatous inflammation. The use of intraarticular platelet-rich autoplasma therapy resulted in a reduction of degenerative and dystrophic changes in the cartilage, an improvement of the tinctorial properties of the articular matrix.

Keywords: osteoarthritis, articular cartilage, platelet-rich autoplasma, knee, experimental study.

Introduction

Deforming osteoarthritis is a heterogeneous group of joint diseases of various etiologies, but with identical biological, morphological and clinical signs and outcome associated with the loss of hyaline cartilage and concomitant damage to other anatomical structures and tissues of the joint (subchondral bone, synovial membrane, ligaments, joint capsule, periarticular tendons). and muscles).

Hyaline cartilage contains a relatively small number of cells surrounded by a large amount of extracellular matrix. Chondrocytes are involved in the regulation of the synthesis and degradation of cartilage matrix components, and these processes are normally in balance [5]. Under the influence of many factors, the balance of degradation and repair processes is disturbed, which subsequently causes the development of osteoarthritis, which manifests itself as degenerative-dystrophic changes in the structure of hyaline cartilage and subchondral bone, inflammation in the surrounding soft tissues, and a violation of the physicochemical properties of the synovial fluid [5, 9].

The possibility of controlling the biological potential of one's own body and using it in the treatment process seems to be very promising and has already been confirmed in a number of works on platelet-rich plasma (PRP). The content of a large number of growth factors in PRP, which can be simultaneously or gradually released into the surrounding tissues, suggests the possibility of influencing the course of the inflammatory process in the joint and hyaline cartilage remodeling [2, 9]. Existing experimental studies on this subject remain debatable and do not yet allow a holistic view of the pathomorphosis of structural changes in cartilage tissue after the use of PRP against the background of osteoarthritis [4, 7, 8].

To assess morphological changes in the structure of hyaline cartilage of the knee joint in experimental osteoarthritis after intra-articular injection of PRP.

Materials and methods

The material for the experimental study was 30 mature Wistar rats weighing (250 ± 2.2) g. The laboratory animals were divided into 3 groups of 10 animals each (2 experimental and 1 control). All manipulations in animals of the 3rd group were performed under general anesthesia using the

ISSN 2792-3983 (online), Published under Volume: 2 Issue: 4 in April-2022

drug "Rometar" according to the method described by the manufacturer. Osteoarthritis was modeled in two experimental groups by intra-articular injection of a 10% suspension of sterile talc [1].

30 days after the modeling of osteoarthritis, the animals of the experimental group No. 2 underwent a double intra-articular injection of 0.2 ml of PRP with a frequency of 1 time in 21 days [9].

Animals of the control group underwent a single intra-articular injection of 0.2 ml of 0.9% NaCl solution. All intra-articular injections were made from a standard anterointernal approach to the left knee joint.

One month after the intra-articular injections, the animals were withdrawn from the experiment by administering a lethal dose of rometar, and the left femur was isolated for subsequent morphological studies.

Cartilage tissue with subchondral bone was fixed in 10% neutral buffered formalin solution (pH 7.4) for 24 hours (Newell K. J., et al., 2001). Acid-free decalcification was carried out in a standard concentration sodium ethylenediaminetetraacetate solution. After complete removal of the mineral component from the bone tissue, standard histological examination was performed using alcohols of increasing concentrations and the preparations were embedded in paraffin, after which sections were made 6–8 microns thick, stained with hematoxylin and eosin according to Mallory (Kiyasov A.P., 2001; Korzhevsky D. E., 2005).

Photorecording of microscopic changes was performed using a complex including an Axio Scope microscope (Carl Zeiss, Germany) and a Power Shot digital camera (Canon, Japan).

Morphometric analysis was carried out using the Video TestMorpho-4 computer program (Russia). To assess the morphological parameters, the thickness of the articular cartilage (L, μm) and the volume fraction of chondrocytes relative to the matrix (OD, %) were determined.

The experimental results were processed by the methods of basic statistical analysis using the Video Test-Morpho-4 (Microsoft, USA) and STATISTICA 6.0 (Stat Soft Inc., USA) programs.

The analysis of parameters with a normal distribution of values was carried out using the Student's t-test, the analysis of non-parametric quantitative traits was performed using the Mann-Whitney test. The $\chi 2$ and Fisher criteria were used to compare qualitative traits. Differences were considered significant if the error probability did not exceed $p < 0.05$.

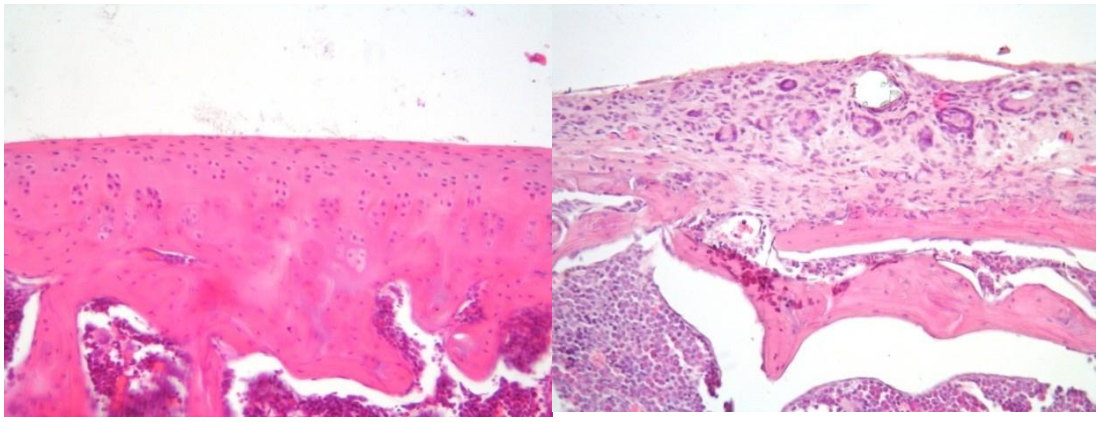
Discussion and results

The study showed that in the control group of animals, the articular hyaline cartilage had a thickness of (330 ± 17.3) μm and a characteristic histological structure. Superficial chondrocytes were characterized by a flattened shape and were located singly in the cartilaginous matrix. Chondrocytes of the transitional and basal zones had a rounded shape and were located in isogenic groups in rows oriented perpendicular to the articular surface. The volume fraction of chondrocytes was (13.7 ± 1.1)% (Table 2). Morphological signs of degenerative-dystrophic processes were not visualized (Fig. 1a). An immunochemical reaction according to Mallory revealed a uniform arrangement of collagen fibers, the absence of foci of ossification (Fig. 2a).

ISSN 2792-3983 (online), Published under Volume: 2 Issue: 4 in April-2022

IJDIAS International Journal of Discoveries and Innovations in Applied Sciences

| e-ISSN: 2792-3983 | www.openaccessjournals.eu | Volume: 2 Issue: 4



After modeling osteoarthritis, there was a decrease in the thickness of the articular cartilage to (121 ± 20.4) μm ($p < 0.05$) and a decrease in the volume fraction of chondrocytes to (1.2 ± 0.6)% ($p < 0.05$). In all zones, multiple "empty lacunae" and chondrocytes with karyopyknosis, extensive areas of destruction of the articular surface with proliferation of connective tissue were noted, in the thickness of which granulomatous inflammation was determined with severe histiomacrophage infiltration and giant multinucleated cells such as foreign bodies, plethora of blood vessels and uneven swelling of the intercellular substances (Fig. 1b).

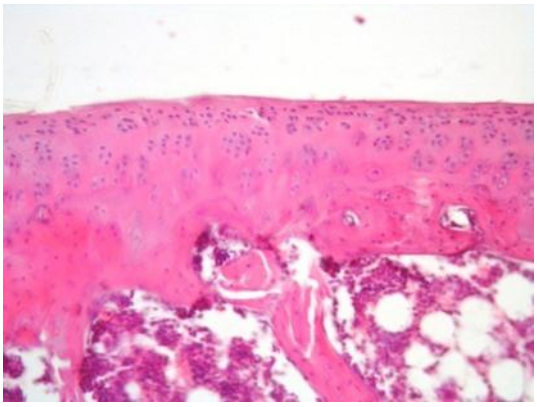
Histochemical examination of the articular cartilage showed uneven staining of collagen fibers with a pronounced violation of the tinctorial properties of the cartilage matrix. In areas of sclerosis, collagen fibers were most intensely stained (Fig. 2b).

After the introduction of PRP against the background of experimental osteoarthritis, an increase in the thickness of the articular cartilage to (275 ± 18.9) μm ($p < 0.05$) and an increase in the volume fraction of chondrocytes to (18.4 ± 2.0)% ($p < 0, 05$).

There were three zones delimited from each other with degenerative changes typical for osteoatrosis, but less pronounced. In the superficial zone, the contours of the articular surface looked even.

Despite the presence of "empty" gaps and chondrocytes with signs of decay and the formation of apoptotic bodies, an increase in the number of both separately located chondrocytes and their isogenic groups in all zones was determined (Fig. 1c).

In the intermediate zone, focal ossification of the intercellular substance occurred, which was especially noticeable when stained according to Mallory. The even distribution of collagen fibers and the tinctorial properties of the cartilage matrix were preserved in all zones (Fig. 2c).



Rice. 1. Different severity of degenerative-dystrophic changes in articular hyaline cartilage in rats of experimental groups.

Stained with hematoxylin and eosin. Magnification ×200:

a – control group;

b – Experimental group No. 1;

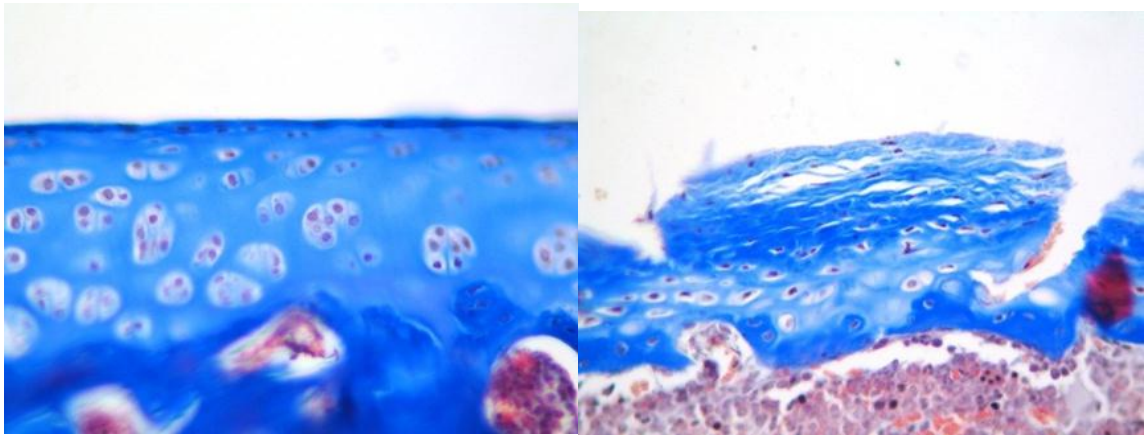
c – Experimental group No. 2.

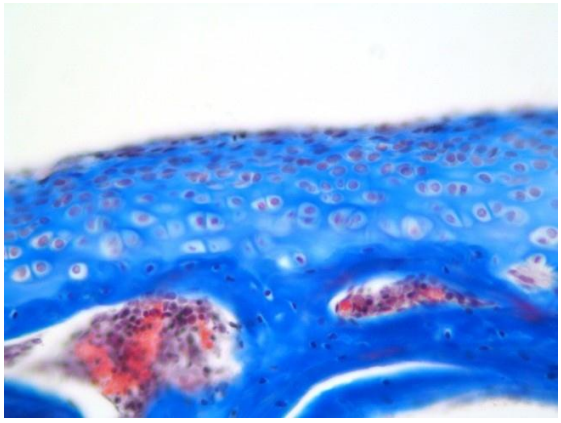
ISSN 2792-3983 (online), Published under Volume: 2 Issue: 4 in April-2022

IJDIAS International Journal of Discoveries and Innovations in Applied Sciences
| e-ISSN: 2792-3983 | www.openaccessjournals.eu | Volume: 2 Issue: 4

Morphometric parameters of articular hyaline cartilage in animals of experimental groups

EXPERIMENTAL GROUPS	THICKNESS, MICRONS	VOLUME FRACTION OF CHONDROCYTES, %
Control	330 ± 17.3	13.7 ± 1.1
Experimental No. 1	121 ± 20.4*	1.2 ± 0.6*
Experimental No. 2	275 ± 18.9**	18.4 ± 2.0**





Rice. 2. Changes in tinctorial properties cartilage matrix in rats experimental groups. Mallory coloring. Magnification x400:

a – control group;

b – experimental group No. 1;

c – experimental group No. 2

The occurrence and progression of gonarthrosis is due to structural changes in the articular cartilage and other tissues of the knee joint. In normal articular cartilage, the processes of destruction and repair of tissues occur rather slowly, are strictly controlled, are in balance, and are the basis of physiological remodeling [5].

To date, in clinical practice, various attempts are being made to influence the course of the inflammatory process in the joint and degenerative-dystrophic changes in hyaline cartilage.

The role of platelets in the pathogenesis of osteoarthritis seems to be more multifaceted than we imagine today. Platelets contain a large amount of rapidly released substances that are involved in the first phase of inflammation, affecting the course of the inflammatory process in the joint, modulating its duration and activity. Platelets activate the processes of migration and activation of leukocytes, as well as repair in tissues, which determine the prospects for the wide use of dosage forms containing them in clinical practice.

ISSN 2792-3983 (online), Published under Volume: 2 Issue: 4 in April-2022

High levels of beta-thromboglobulin, platelet factor 4 in PRP stimulate an inflammatory response by activating neutrophil migration. At the same time, PRP may resolve inflammation by restoring the endothelial barrier by releasing hepatic growth factor, VEGF, and TGF-b [6, 10]. The release of biologically active substances from platelet granules, on the one hand, inhibits the activity of metalloproteinases, and on the other hand, stimulates proliferation, which causes the chondroprotective effect of PRP. It is also known from the literature that in vitro PRP stimulates the production of type II collagen by chondrocytes and reduces the level of chondrocyte apoptosis [7].

The results obtained do not conflict with theoretical data on the effect of HA as an inhibitor of exudation, the formation of pro-inflammatory mediators, and as a component of matrix metabolism. The use of PRP, which has a dual effect on both pro- and anti-inflammatory cytokines, and significantly increases the concentration of various growth factors, in the shortest possible time increases the proliferative activity of granulation tissue and leads to the activation of damaged cartilage tissue, thus resolving the inflammatory process.

Conclusion

When modeling osteoarthritis in the knee joint in mature Wistar rats, gross structural changes occur in the articular cartilage, up to its complete destruction, accompanied by vascular proliferation and granulomatous inflammation.

The introduction of PRP against the background of developed osteoarthritis is accompanied by a decrease in the severity of degenerative-dystrophic changes, an improvement in the indicators of the tinctorial properties of the articular cartilage matrix.

References

1. Котельников Г. П., Ларцев Ю. В., Махова А. Н. Сравнительная оценка структурных изменений тканей сустава при различных моделях экспериментального артроза // Казанский медицинский журнал. – 2006. – № 1. – С. 31–35.
2. Обогащенная тромбоцитами аутологичная плаз- ма в лечении пациентов с гонартрозом III стадии / Д. А. Маланин, В. В. Новочадов, С. А. Демкин и др. // Травматология и ортопедия России. – 2014. – № 3. – С. 52–59.
3. Andia I., Sanchez M., Maffulli N. Joint pathology and platelet-rich plasma therapies // Expert Opin. Biol. Ther. – 2012.– Vol. 1, № 1. – P. 7–22.
4. Guner S., Buyukbebeci O. Analyzing the effects of platelet gel on knee osteoarthritis in the rat model. Clin. Appl. Thromb. Hemost. – 2013. – Vol. 19. – P. 494–498.
5. Hedbom E., Hauselmann H. J. Molecular aspects of pathogenesis in osteoarthritis: the role of inflammation // Cell. Mol. Life Sci. – 2002. – Vol. 59. – P. 45–53.
6. Y. T. Lin, B. L. Chiang, et al. // Osteoarthr. Cartil. – 2006. – Vol. 12. – P. 1237–1247.
7. Influence of platelet-rich plasma on chondrogenic differentiation and proliferation of chondrocytes and mesenchymal stem cells / A. Drengk, A. Zapf, E. K. Stur- mer, et al. // Cells Tissues Organs. – 2009. – Vol. 189, № 5. – P. 317–326.
8. Kwon D. R., Park G. Y., Lee S. U. The effects of intra-articular platelet-rich plasma injection according to the severity of collagenase induced knee osteoarthritis in a rabbit model // Ann. Rehabil. Med. – 2012. – Vol. 6, № 4. – P. 458–465.

ISSN 2792-3983 (online), Published under Volume: 2 Issue: 4 in April-2022

9. Platelet-rich plasma: why intra-articular? A sys- tematic review of preclinical studies and clinical evidence on PRP for joint degeneration / G. Filardo, E. Kon, A. Roffi, et al. // Knee Surg. Sports Traumatol. Arthrosc. – 2015. – Vol. 23. – P. 2459–2474.
10. Walshe T. E., Dole V. S., Maharaj A. S. R. Inhibi- tion of VEGF or TGF-beta signaling activates endothelium and increases leukocyte rolling // Arterioscler. Thromb. Vasc. Biol. – 2009. – Vol. 29. – P. 1185–1192.
11. Amrilloevich N. D. Features of the application of external osteosynthesis in gonarthrosis //Asian journal of Pharmaceutical and biological research. – 2021. – Т. 10. – №. 2.
12. Ziyadullaev A., Nurmonov S., Parmonov A. Study of the catalytic reaction of acetylene with cyanuric acid //Journal of science. Lyon. – 2020. – №. 8-1. – С. 11-14.
13. Amrilloevich N. D. et al. INTRA-ARTICULAR PLATELET-RICH PLASMA INJECTIONS INTO THE KNEE IN PATIENTS WITH EARLY OSTEOARTHRITIS //Asian journal of pharmaceutical and biological research. – 2021. – Т. 10. – №. 3.
14. Uli Z. A. K., Amrilloevich N. D. MORPHOLOGICAL CHANGES IN THE HYALINE CARTILAGE OF THE KNEE JOINT AGAINST THE BACKGROUND OF INTRA-ARTICULAR ADMINISTRATION OF THE PREPARATION OF HYALURONIC ACID IN RATS WITH EXPERIMENTAL OSTEOARTHRITIS //Asian journal of pharmaceutical and biological research. – 2021. – Т. 10. – №. 3.
15. Qizi R. G. B. OZBEK FOLKLORIDA TOL OBRAZINING O'ZIGA XOS TALQINLARI //НамДУ илмий ахборотномаси. – 2020. – Т. 1. – С. 5,311-319.
16. Bahronovna R. G. Epic Interpretations of the Image of Trees in Uzbek Folk Tales //European Journal of Life Safety and Stability (2660-9630). – 2021. – Т. 12. – С. 330-334.
17. Рустамова Г. Б. HISTORICAL-MYTHOLOGICAL BASES OF IMAGES ASSOCIATED WITH TREES IN FOLKLORE.
18. Шербекова Г. Я. АФСОНА ВА РИВОЯТЛАРДА “ҲАЁТ ДАРАХТИ” ОБРАЗИ ИФОДАЛАНИШИГА ОИД АЙРИМ МУЛОҲАЗАЛАР //Scientific progress. – 2022. – Т. 3. – №. 2. – С. 244-250.
19. Uktamovich H. B., Shavkatovich A. S. OPTIMIZATION OF BLOCKING INTRAMEDULLARY OSTEOSYNTHESIS METHODS FOR FEMORAL FRACTURES //Asian journal of pharmaceutical and biological research. – 2021. – Т. 10. – №. 3.
20. Mirzamurodov H. H. et al. OPTIMIZATION OF TOTAL HIP ARTHROPLASTY FOR DYSPLASTIC COXARTHROSIS //Новый день в медицине. – 2020. – №. 4. – С. 667-672.

ISSN 2792-3983 (online), Published under Volume: 2 Issue: 4 in April-2022