

REPARATIVE OSSEO- AND CHONDROGENEZ IN THE CONDITIONS OF INDUCED OSTEOARTHRISIS IN LABORATORY ANIMALS

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Keywords: arthropathy, reparative chondrogenesis, joint, articular cartilage.

Abbreviations: OA – osteoarthritis

Introduction

Evaluation of the arthropathy pathomorphism of different etiologies and developing on this basis, the ways to improve the reparative properties of the damaged tissues of the joint still remains one of the actual tasks of clinical morphology and veterinary medicine. The most vulnerable is the knee joint, one of the most functionally loaded and, thus, having morphological complexity of the organization [2,5,6].

It is known that the joint is a multi-component system, besides all joints are kinematic pairs those constructed with locomotive osteal elements constructed with articulated bones contacting between each other. These kinematic pairs are fixed with ligaments, covered with an articular cartilage in the place of contact and enclosed by fibrotic articular capsule in a sealed cavity. The internal surface of this capsule is presented with synovial membrane, which blood and lymphatic vessels are producing a synovial fluid [6]. Between this cavity and the "internal environment" (blood and lymphatic vessels of the capsule's synovial membrane) different metabolic processes continuously run. They regulate the amount, composition and properties of synovial fluid respectively to dimension and character of loading undergoing by the joint.

Therefore, joint synovial environment, determined by the kind of relationship between the capsule's synovial membrane and synovial fluid is one of the major factors that provide reliable tribomechanical conditions into the junction. The distortion of these processes is at the heart of many arthropathies [1,2,4,5].

Objective

To evaluate the complex of structural changes in synovial tissues of experimental models (rats) at the state of induced gonarthrosis. To assess the chondrogenous resources and lubricative properties of the intra-articular injection material Noltrex, intended for replacement of synovial fluid.

Materials and Methods

Studies were conducted on the basis of the Animal Anatomy and Histology Department (n.a. A.F. Klimov) of Moscow State Academy of Veterinary Medicine and Biotechnology n.a. K.I. Scryabin. Experimental animals were 27 rats weighting 200 - 250 g. There were dissected the left knee joint cavity of animals in aseptic and antiseptic condi-

tions under aether general anesthesia and were mechanically created wedge-shaped defect on the surface of the articular cartilage of the femur. Surgical wound had been sewn up layer-by-layer.

Vivarium conditions, content, care, experimental research, surgical intervention and withdrawal from an experiment (euthanasia) were carried out in accordance with the requirements, governing the humane management of laboratory animals (Good Veterinary Practice). Those are the Directive of the Department of Health of USSR № 755 dated 12.08.1977, № 701 dated 27.07.1978, № 1179 dated 10.10.1983 and the Directive of the Department of Health of RF № 267 dated 12.06.2003, "Sanitary rules on provisions of the necessary facilities, equipment and maintenance of experimental biological clinics (vivariums)", "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes"(1986) and "Declaration of Helsinki of the World Medical Association"(1964).

7 days after surgery 3 animals were removed from the experiment under anesthesia by dislocation of the cervical vertebrae to assess post-traumatic alterations occurring into the joint. The other experimental animals were divided into 2 groups of 12 animals each:

- the animals of the first (control) group were injected with an indifferent substance - 0.2-0.3 ml of sterile isotonic sodium chloride solution;
- animals of the second (experimental) group received intra-articular injection of Noltrex in equal volume.

Animals of both groups were removed from the experiment in two weeks and one month after material administration. The species of operated joints were taken within diaphysal region of the femur, selected tissue material was fixed in 10% neutralized formalin, then dehydrated in a series of ethanol dilutions and histological sections were prepared using standard procedures. Paraffin sections with thickness of 5-6 microns were stained using Mayer's hematoxylin - eosin, and viewed using a microscope Nikon.

Results

The result of primary clinical and morphological studies has shown that designed defect of cartilage surface and subchondral bone tissue could be classified as OA. This is confirmed by joint stiffness, enlargement of joint's volume and irregularity of cartilage space on a survey X-ray of the joint. Articular cartilage is irregular in thickness, dramatically get thinned in site of experimental trauma. It is characterized by presence of microdefects in the form of longitudinal and transverse fissures of different length, gaps and matrix fibrillization (Fig. 1).

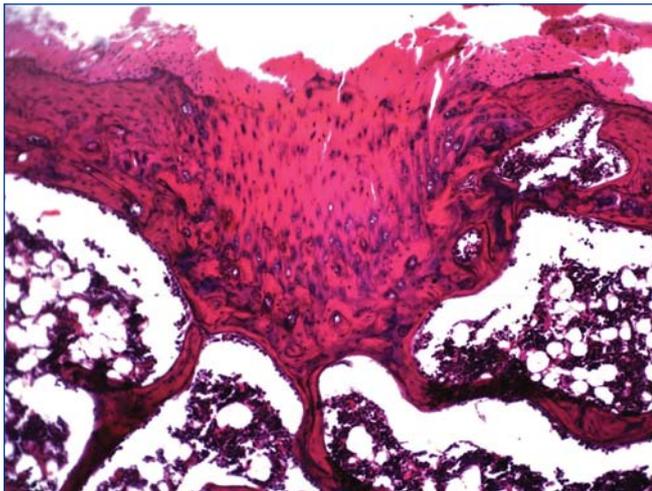


Fig. 1. Histologic specimen. Structural changes of the articular cartilage at induced OA. (See text.) Hematoxylin and eosin, obj.x10, oc.x10 (x100).

In general, the articular cartilage showed changes of position-specific distribution of cells into the area of micro-defects, leading to a violation of cytological architectonics. After two weeks of observation in animals of control group there was detected a decomposition of structural zones into the articular cartilage, also a blood vessels invasion towards the area of uncalcified cartilage, local disturbance of the tide mark integrity. Reparative reactions of intra-articular and periarticular tissues we haven't detected (Fig. 2, a).

After a month in the cartilage covering a violation of zonal differentiation progressed. We revealed a decrease in chondrocyte quantitative representation and their simultaneous focal proliferation and breakthrough of the edge of cartilage mineralization (Fig. 3, b).

In animals of the experimental group the influence of material Noltrex on a structural and functional cartilage state was evaluated in the dynamics of regenerative process. Two weeks after material injection there were noticed the structural regenerative transformation of the articular cartilage leading to restoration of local articular surface.

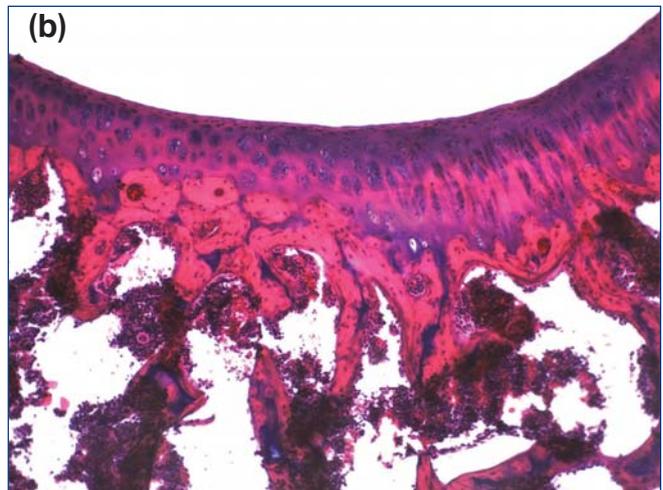
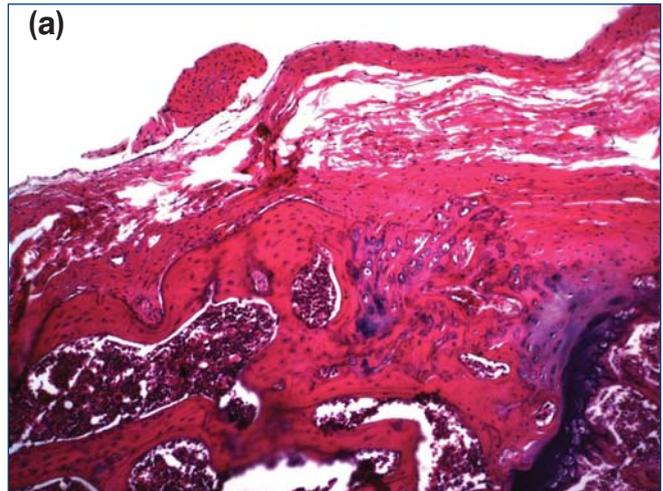


Fig. 2. Histologic specimen. The structural state of the articular cartilage at the 14th day in control (a) and experimental (b) groups. Hematoxylin and eosin (x100).

Regenerate was represented by differentiated chondrocytes without positional specificity, and in the subchondral bone we revealed osteogenesis centers. The surface area of the cartilage acquired a specificity of structural organization of hyaline cartilage tissue (Fig. 2, b).

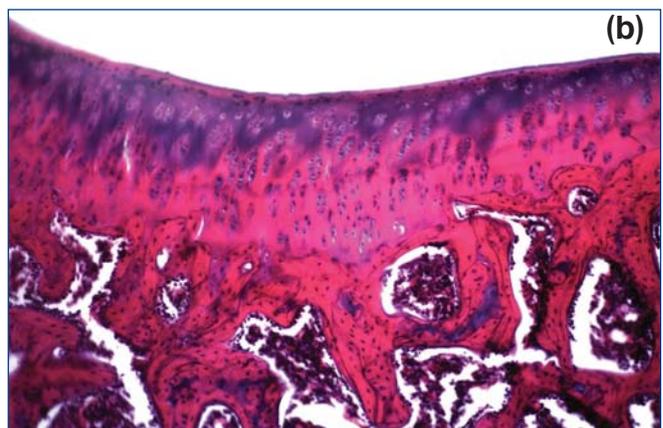
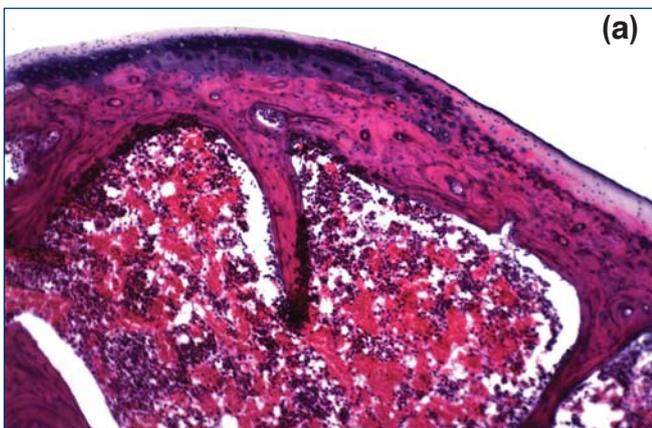


Fig. 3. Histologic specimen. The structural state of the articular cartilage at the 30th day in control (a) and experimental (b) groups. Hematoxylin and eosin (x100).

A month later, manifestation of reparative chondrogenesis expressed in the restoration of the specificity of chondrocytes and cartilage matrix distribution, which was accompanied by the alignment of the contours of the articular surface and formation of entire coverage. A presence of pronounced osteochondral junction may reflect the recovery of subchondral bone's trophic potential. Thus administration of material led to the restoration of structural organization of articular cartilage after the development of mechanically induced OA and restoration of disturbed relations between the cartilage and synovial environment of the joint (Fig. 3, b).

Conclusions

Material Noltrex improves reparative chondro- and osteogenesis in damaged tissues of a knee joint, as evidenced by the formation of entire joint coverage with organ specific articular zonal differentiation. As well as centers of osteochondral junction, indicating a restoration of

relationship between calcified zone of articular cartilage and subchondral bone. Since restoration of structural state of osteochondral junction into the articular cartilage is one of the most important attribute of joint synovial environment normalization, we can conclude that injection material Noltrex designed for substitution of synovial liquid does really compensate its lubricative features and optimize tribomechanical conditions into the joint cavity.

Based on preclinical testing data Noltrex can be recommended for widespread use in the practice of veterinary medicine in the treatment of animals with various etiology arthrosis (posttraumatic and dysplastic nature).

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SUMMARY

N.A. Slesarenko, E.O. Shirokova. Reparative osseo- and chondrogenez in the conditions of induced osteoarthritis in laboratory animals. It is shown, that use of the drug noltrex led to the restoration of structural organization of the articularis cartilage after development induced by mechanical means of osteoarthritis and restoration of the disturbed relationship between the cartilage and synovial medium of the joint.