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Voytsenko K.I.
Danylo Halytskyi Lviv National Medical University, Department of Normal Anatomy, Lviv, Ukraine, kostya_89@ukr.net

PATHOLOGIC SIGNS IN THE CARTILAGINOUS LAYER STRUCTURES OF THE KNEE JOINT OF RATS AT THE END OF THE SECOND WEEK OF EXPERIMENTAL OPIOID EFFECT

Abstract. *Objective of the study was to investigate the results of two-week experimental opioid analgesic effect on the dynamics of changes in the cellular components of the knee joint cartilaginous layer in rats. The objective was implemented by means of microscopic methods resulting in obtaining serial sections of the knee joint cartilaginous layer components with visualized pathomorphologic signs of experimental two-week opioid effect. Microstructural specimens were prepared according to the common methods using staining agents such as hemtoxylin, eosin, azure and azan according to Handenhein's method. The experimental examination performed by us resulted in determination the fact that at the end of the fourteenth day the signs of degenerative-dystrophic changes appeared. Acellular lamina structures were destructed and broken down into fibers, and the areas of vertical microcracks were formed with appearance of usures. At this term of the experiment vacuole dystrophy of chondrocytes occurred associated with necrotic changes. The epiphyseal layer thickness was heterogeneous, with preserved height in some places. Hyperemia of the fine venous and less arterial vessels was found in the synovial membrane. The results of the conducted experimental examination will enable to form further image concerning the dynamics of increasing pathomorphologic signs in the cellular elements of the knee joint cartilaginous layer at different terms of experimental opioid effect.*
Key words: *experimental opioid effect, cartilage, knee joint, rat.*

Introduction. The cartilaginous layer of the knee joint osseous elements is a flexible-elastic material with a high organization level consisting of chondrocytes, water and extra-cellular matrix. These elements are known to lack vascularization, lymph outflow and innervation. The source of chondrocyte trophic is synovial fluid [1]. Changes of the quantitative or qualitative content of the synovial fluid promote the onset of degenerative processes [2]. A number of investigations deal with the issues of uncontrolled administration of drastic and psychotropic drugs of medical and surrogate origin [3, 4]. There is a number of studies dealing with a toxic effect of opioids on the body organs and systems on the experimental level [5 - 8]. But even now the available home and foreign literature does not contain issues which substantially and systematically explain the interrelations between opioid etiological effects on pathomorphologic changes in the morphological organization of the cartilaginous layer cellular elements of the knee joint osseous elements. This fact promoted us to investigate the issue dealing with examination of peculiarities of pahtomorphologic changes in the microstructural organization of the knee joint cartilaginous layer at the end of the two-week experimental opioid effect.

Objective: to investigate the results of two-week experimental opioid analgesic effect on the structural organization of the knee joint cartilaginous layer in rats.

Materials and methods. Material of the study was 16 mature outbred male rats with the body weight of 92 g, and 4,5 months of age. The animals were i/m injected with Nalbuphine every day once a day at the same period of time (10-11 a.m.) during 14 days. The initial dose of Nalbuphine was 8 mg/kg during the first day, and 15 mg/kg during the second day. Therefore, conditions of chronic opioid effect were created [9].

The animals were divided into two groups. The 1st group of animals received Nalbuphine during 14 days at the same period of time (10-11 a.m.) with the following taking the material for examination (at the end of the 2nd week of experimental opioid effect); the 2nd control group received i/m injections of physiological solution during 14 days at the same period of time (10-11 a.m.).

All the animals were kept in vivarium and the work concerning their care, marking and all the other manipulations were conducted according to the regulations of "The European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" [Strasburg, 1985], "Ethical Principles and

Guidelines for Experiments on Animals", approved by the First National Congress on Bioethics [Kyiv, 2001]. The Bioethics Expert Board of Danylo Halytskyi Lviv National Medical University determined that conducted scientific studies correspond to ethical requirements according to the Order of the Ministry of Health of Ukraine № 231 dated 01. 11. 2000 (minutes № 10 dated 26.12. 2011).

Before the material for biopsy was taken an animal was put to sleep by means of the intraperitoneal introduction of Thiopental (in the dose of 25 mg/kg). The following material for the microstructural examination was used: distal epiphysis of the femoral and proximal epiphysis of the tibia of rats considering integrity of the topographic ratio of the knee joint structural component. After decalcification histological specimens were prepared according to the common methods using staining agents hematoxylin, eosin, azure and azan according to Handenhein's method [10].

Results. Microstructural examination of the layer-by-layer cellular organization of the knee joint cartilaginous layer in the experimental group of animals at the end of the 14th day of opioid effect resulted in finding marked degenerative-dystrophic changes in the knee joint cartilage. Acellular lamina structures were destructed and broken down into fibers (Fig. 1). In some places the areas of formation of vertical microcracks were found, and in the peripheral areas of the cartilaginous surface usures were formed. Synovial fluid penetrated into certain microcracks of the articular surface. Destruction of the main substance in the tangential area was determined associated with its breaking down into fibers, appearing focal necrotic changes in chondrocytes. In certain areas of the tangential zone, transitional and radial zones the cartilaginous matrix increased in volume; its heterogeneous, sometimes intensively acidophilic staining was detected (Fig. 2) which is indicative of changes of tinctorial properties of the major cartilaginous substance.

The amount of acid glycosaminoglycans decreased in the matrix of the tangential and transitional areas. Heterogeneous focal increase of the amount of acid glycosaminoglycans occurred in the thickened acellular lamina and in the matrix of the tangential zone (Fig. 3). The level of acid glycosaminoglycans decreased in the matrix of the transitional and radial areas. Chondrocytes of the tangential area underwent necrotic changes. Lacunas without chondrocytes were often visualized. Collagen fibers, especially in the tangential area, and less in deeper located areas, lose their architectonics, became edematous unevenly, broke down into separate fragments, and underwent lysis. Chondrocytes of the transitional area rarely formed isogenic groups. Vacuole dystrophy of chondrocytes occurred associated with necrotic changes, especially often found in the transitional and less often in the radial areas. In case of vacuole dystrophy chondrocytes became enlarged in their volume, edematous; their cytoplasm was clarified considerably and contained vacuoles filled with light fluid. Rather often vacuoles assimilated. Under conditions of development of necrotic changes the chondrocyte nucleus became smaller in the volume, stained evenly, intensively basophilic (karyopicnosis). Sometimes the nucleus was dissolved and was not visualized (karyolysis). The cytoplasm of these cells was sharply cleared. Certain chondrocytes were completely broken down. The membranes of neighboring chondrocytes were destructed, and small areas were formed in their places in the major cartilaginous substance and visualized which were filled with cleared cytoplasmic fluid.

Single proliferating chondrocytes of the transitional area underwent dystrophic and necrotic changes. The basophilic line was broken down; it was not visualized in the majority of

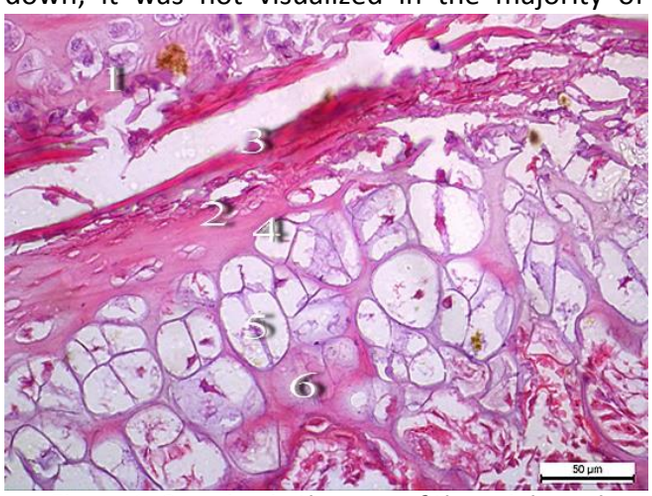

Figure 1. Covering in the area of the epiphyseal cartilage of the femoral bone distal end and surfaces of the patella in rats at the end of the 14th day of opioid effect. Stained with hematoxylin and eosin. Microphotograph. Magnification x 400. 1 –patella; 2 – femoral bone ; 3 – destruction, breaking down into fibers and microcracks of the acellular lamina ; 4– vacuole dystrophy and necrosis of chondrocytes of all the areas of the articular joint ; 5 – chondrocyte-free lacunas; 6 – increased volume and uneven staining of the cartilaginous matrix

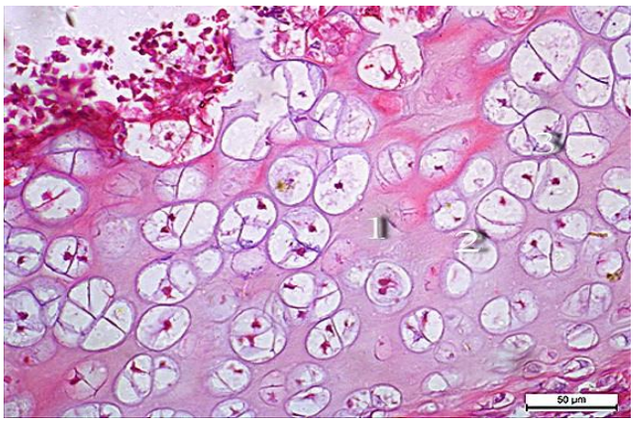

Figure 2. Covering in the area of the epiphyseal cartilage of the femoral bone distal end in rats at the end of the 14th day of opioid effect. Stained with hematoxylin and eosin. Microphotograph. Magnification x 400. 1– increased volume and uneven staining of the cartilaginous matrix; 2– marked vacuole dystrophy and necrotic of chondrocyte changed of all the areas of the articular joint; 3 – chondrocyte-free lacunas.

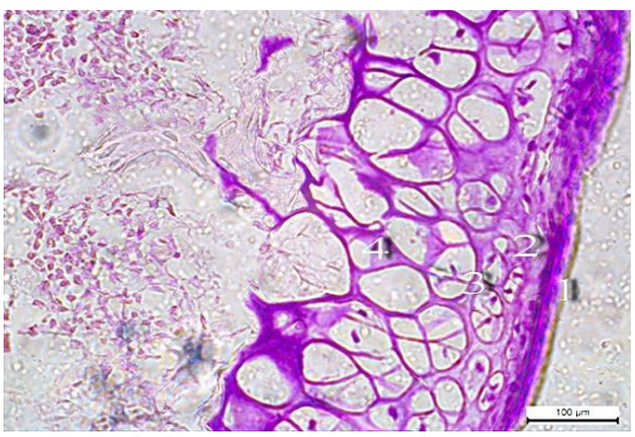

Figure 3. Covering in the area of the epiphyseal cartilage of the femoral bone distal end in rats at the end of the 14th day of opioid effect. Stained with azure. Microphotograph. Magnification x 200. 1 – uneven enlargement of acid glycosaminoglycans in the thickened acellular lamina; 2 – uneven enlargement of the amount of acid glycosaminoglycans in the tangential area; 3 – decreased amount of acid glycosaminoglycans in the matrix of the transitional area; 4 – decreased amount of acid glycosaminoglycans in the matrix of the radial area.

articular surface areas; only its residues were found in some places. In the central areas of the subchondral zone the amount of the osseous tissue was inconsiderable; in some places unevenly stained, thinned osseous beams were visualized. In the peripheral zones uneven accumulation of non-mineralized osteoid was found. The epiphyseal cartilage thickness was uneven. Certain areas with sharply thinned uneven epiphyseal lamina of growth were found (Fig.4). In such places only 6-9 lines of chondrocytes were visualized. Severe phenomena of vacuole dystrophy developed there with alternation of necrotic changes. Multiplication of chondrocytes in proliferation zone was weak; short cellular columns were rarely formed. In some places the areas with thicker epiphyseal cartilage were visualized (Fig. 5), with clear area distribution and formed columns of proliferating chondrocytes.

Certain areas were present where enlargement and uneven staining of the major substance of the cartilaginous matrix were determined. Newly formed osseous beams were rarely found. In the matrix of the rest zone uneven enlargement of acid glycosaminoglycans was present (Fig. 6). At the same time, the areas of focal decrease of the content of acid glycosaminoglycans were determined in other zones of the epiphyseal

Figure 4. Sharp decrease of the epiphyseal cartilage thickness in the femoral bone end of the tibia of a rat at the end of the 14th day of opioid effect. Stained with hematoxylin and eosin. Microphotograph. Magnification x 400. 1 – single short columns of chondrocytes; 2 – dystrophic and necrotic changes of chondrocytes.

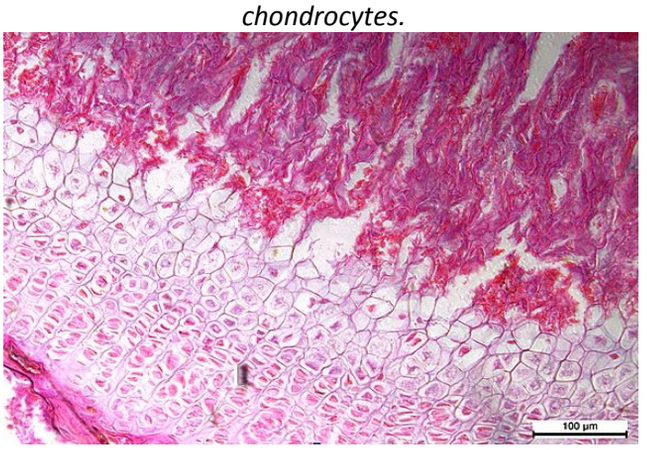

Figure 5. Cartilaginous layer in the area of the epiphyseal cartilage in the proximal end of the tibia of a rat at the end of the 14th day of opioid effect. Stained with hematoxylin and eosin. Microphotograph. Magnification x 400. 1 – formed volumetric columns of chondrocytes in the proliferation area.

cartilage, especially in the area of destruction. Hyperemia of the small venous and less arterial vessels was detected in the synovial fluid. The lumens of the dilated vessels in addition to erythrocytes contained lymphocytes and single neutrophils. The major substance of the connective tissue around dilated vessels was edematous, contained small infiltrations, manifested mainly by lymphocytes, macrophages, tissue basophils and single neutrophils.

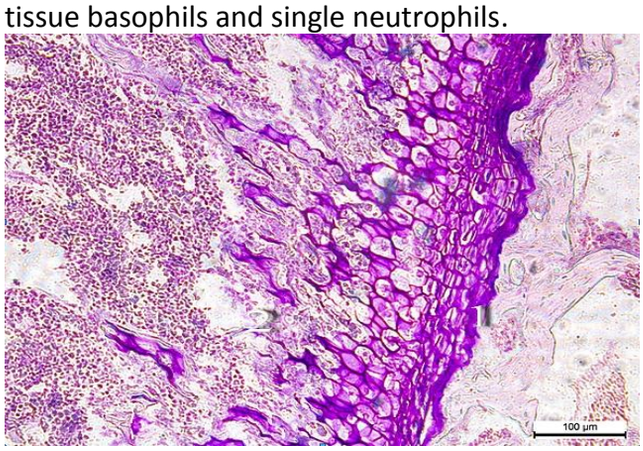

Figure 6. Covering in the area of the epiphyseal cartilage in the distal end of the femoral bone of a rat at the end of the 14th day of opioid effect. Stained with azure. Microphotograph. Magnification x 200. 1– uneven enlargement of the amount of acid glycosaminoglycans in the matrix of the rest area; 2 – focal decrease of the amount of acid glycosaminoglycans in the destruction area.

Certain areas of uneven swelling of the collagen fibers were present. Focal proliferation of synoviocytes was determined resulting in increasing amount of synovial cells in the above mentioned areas; the latter unevenly protruded into the joint cavity.

Prospects of further studies. Pathomorphological changes detected in the cellular structures of the knee joint cartilaginous layer at the end of the 14th day of the experimental opioid effect will further enable to conduct comparative characteristics of appearance and increase of pathological changes as far as the dose of opioid increases and duration of the experiment becomes longer. In future on the basis of the obtained information it will enable to conclude concerning initial changes in the components of the knee joint cartilaginous layer with the aim to perform a correcting effect.

Conclusions: in the result of experimental two-week opioid analgesic effect we have determined that in comparison with preliminary term the processes of increasing pathological changes occurred in the knee joint cartilaginous layer resulting in the appearance of the areas of decreased cartilaginous tissue height both within the limits of the articular cartilage and the areas of epiphyseal cartilage location. These signs are indicative of intensification of destructive-degenerative changes of the articular cartilage.

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