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Dynamics of pathomorphological changes in the structural organization of the intervertebral disc at the end of the seventh and fourteenth day of experimental opioid exposure at the ultrastructural level

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Abstract

In general, the modern literature pays attention to the issues of spine pathology and intervertebral discs. A significant percentage of vertebral disorders - scoliosis, osteochondrosis, spinal disc herniation, etc., occur as a result of exposure to various factors and manifest in changes of the intervertebral discs. **The aim** of our work was to study at the ultrastructural level the features of pathomorphological manifestations in the structural components of the intervertebral disc at the end of the seventh and fourteenth days of experimental opioid exposure. **Materials and methods of research.** The objects of the study

were 32 sexually mature, white, male rats, weighing 92 - 103 g, aged 4.5 months. Animals were injected with nalbupine intramuscularly once daily (at 10-11 a.m.) for 14 days. The initial dose of nalbuphine was 8 mg/kg during the first week, 15 mg/kg during the second week. It created the conditions of chronic opioid exposure. Before sampling, the animals were withdrawn from the experiment using dibutyl ether. Intervertebral discs of rats were used as a material for ultrastructural study. Ultrastructural specimens were prepared according to the accepted methods. **The results of the study.** As a result of the sampling after 7 days of opioid exposure we found inhomogeneous osmiophilicity and compaction of the nucleus pulposus matrix in which intensively accumulated osmiophilic grains of glycogen proteoglycans, increased the number of collagen fibers, some of them were heterogeneous. It was also noted the development of moderate degenerative changes in some notochondral cells, which was accompanied by increased vacuolization of the cytoplasm by inhomogeneous compaction of the nucleus and an increase of heterochromatin there. After 14 days necrotic changes in the cells of the nucleus pulposus, as well as the destruction of collagen fibers of the annulus fibrosus were found. In particular, an increase in the amount of heterochromatin in the nucleui of notochondral cells, which was accompanied by a decrease in the volume of the nucleui and inhomogeneous swelling of the cytoplasm. Active fibroblasts were often visualized in the annulus fibrosus. Intense osmiophilicity and thickening of collagen fibers of the annulus fibrosus were observed in some areas of the fibrous ring. Focal destruction of collagen fibers was also noted. In the areas of destruction the fibrils of collagen fibers disintegrated into an inhomogeneous fine-grained stratified mass and were located loosely. **Conclusions**. At the end of the first week we found that the cytoplasmic processes of chondrocytes decreased in volume, shortened, underwent fragmentation and destruction, some of them detached from the surface of the plasmolemma. At the end of the second week signs of opioid exposure progressed and manifested by an increase in the destruction of cytoplasmic processes in chondrocytes. Also focal destruction of collagen fibers was noted.

Keywords: **opioid; intervertebral disc; rat; seventh and fourteenth day.**

Connection of the study with scientific programs, plans, topics. The article is a part of the research of the Department of Normal Anatomy "Morphofunctional features of internal organs in the pre- and postnatal periods of ontogenesis under the influence of opioids, food supplements, reconstructive surgery and obesity" with the deadline 2020-2024, state registration number 0120U002. The authors are co-performers of the study.

Introduction. In general, the modern literature pays attention to the issues of spine pathology and intervertebral discs. A significant percentage of vertebral disorders - scoliosis, osteochondrosis, spinal disc herniation, etc., occur as a result of exposure to various factors and manifest in changes of the intervertebral discs [1-8].

The role of subchondral bone tissue in the development of osteoarthritis is also known, which is associated with the expression by osteoblasts of proinflammatory cytokines and growth factors that affect chondrocytes and close the cycle of osteoarthritis [9-14]. If these data are extrapolated to the bone tissue adjacent to the intervertebral disc, it is possible that its role is also important in the development of destructive disorders in the disc [10,15-18].

The aim of our work was to study at the ultrastructural level the features of pathomorphological manifestations in the structural components of the intervertebral disc at the end of the seventh and fourteenth days of experimental opioid exposure.

Materials and methods of research. The material of the study were sexually mature, white, outbred rats - males in the amount of 32 and animals weighing 80 - 135 g, aged 4.5 - 7.5 months. Animals were injected intramuscularly with nalbuphine once daily for 14 days at a time (10-11 am). The initial dose of nalbuphine was 8 mg / kg during the first week, 15 mg / kg during the second week. Thus created the conditions for chronic opioid exposure [19].

Animals are divided into 3 groups. The 1st group of animals received nalbuphine for 7 days in one period of time (10 - 11 o'clock in the morning), followed by collection of study material. 2nd group of animals received nalbuphine for 14 days in one period of time (10 - 11 o'clock in the morning), followed by collection of study material; 3rd control group, which for 14 days received injections of saline intramuscularly in one period of time (10 - 11 o'clock in the morning), followed by sampling. All animals were kept in a vivarium, the experiment was conducted in accordance with the principles of bioethics in accordance with the provisions of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986), Council Directive 86/609 / EEC (1986), Law of Ukraine № 3447-IV "On protection of animals from cruel treatment", general ethical principles of animal experiments, approved by the First National Congress of Ukraine on Bioethics (2001), confirmed by the conclusion of members of the commission on bioethics of Lviv National Medical University named after Danylo Halytsky. 10 of December 15, 2019).

Before sampling, the animals were removed from the experiment using dibutyl ether. Intervertebral discs of rats were used as material for ultrastructural study. Ultrastructural preparations were prepared according to the generally accepted method [20-22].

Research results

As a result of the collection of experimental material after 7 days in shur, who were under the influence of opioid analgesic at a dose of 8 mg / kg at the ultrastructural level, we found inhomogeneous osmophilicity and compaction of the matrix of the gelatinous nucleus (Fig. 1, 2)**,** in which intensively osmophilic grains of glycogen proteoglycans accumulated, the number of fibrils of collagen fibers increased, some of them were heterogeneous (Fig. 3).

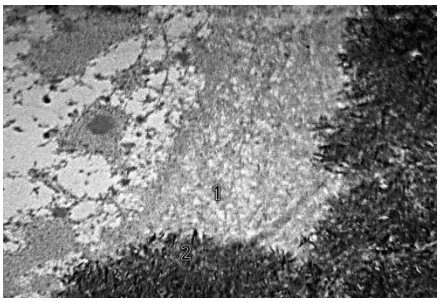

Fig. 1. The gelatinous nucleus after 7 days of opioid exposure. Photomicrograph. Coll. x4000.
1 - heterogeneous osmophilicity; 2 - compaction of the matrix of the gelatinous nucleus

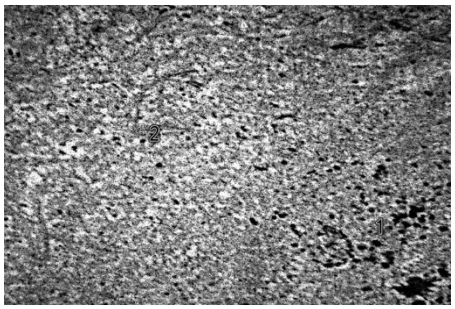

Fig. 2. The gelatinous nucleus after 7 days of opioid exposure. Photomicrograph. Coll. x4000.
1 - heterogeneous osmophilicity; 2 - compaction of the matrix of the gelatinous nucleus.

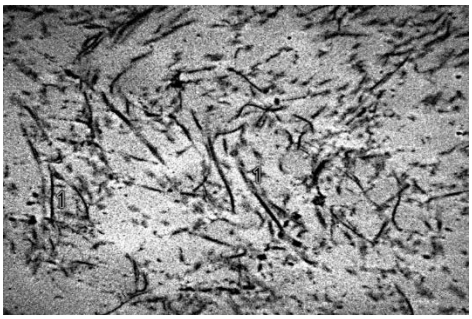

Fig. 3. The gelatinous nucleus after 7 days of opioid exposure. Photomicrograph. Coll. x6000.
1 - inhomogeneous thickening of fibrils of collagen fibers of the matrix of the gelatinous nucleus.

The development of moderate degenerative changes of individual notochondral cells was also registered, which was accompanied by increased vacuolation of the cytoplasm by inhomogeneous compaction of the nucleus, increase in heterochromatin in it. Degenerative changes were registered in chondrocytes, which were accompanied by the appearance of bulky vacuoles in the cytoplasm, filled with inhomogeneous osmophilic content **(Fig. 4, 5).** The cytoplasmic processes of chondrocytes decreased in volume, shortened, underwent fragmentation, destruction, some of them detached from the surface of the plasmolemma **(Fig. 6).**

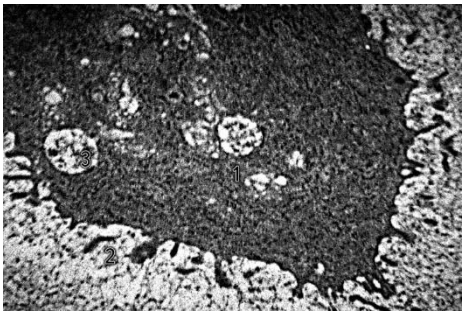

Fig. 4. Degeneration of the chondrocyte nucleus after 7 days of opioid exposure. Photomicrograph. Coll. x4000.

1 - chondrocyte degeneration; 2 - shortening and destruction of cytoplasmic processes; 3 - vacuoles with inhomogeneous osmophilic content in the cytoplasm.

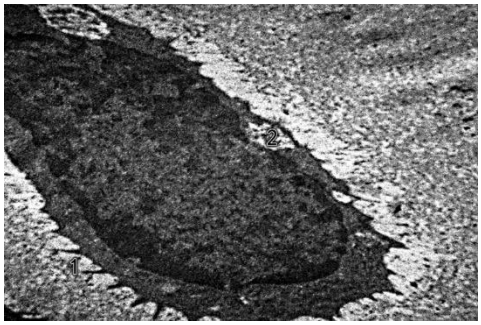

Fig. 5. Chondrocyte degeneration after 7 days of opioid exposure. Photomicrograph. Coll. x4000.

1 - shortening and destruction of cytoplasmic processes; 2 - vacuoles with inhomogeneous osmophilic content in the cytoplasm.

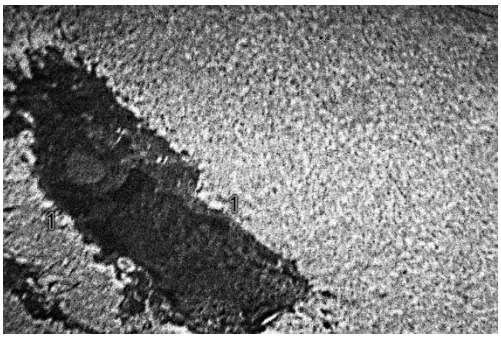

Fig. 6. Chondrocyte degeneration after 7 days of opioid exposure.
Photomicrograph. Coll. x4000.
1 - shortening and destruction of cytoplasmic processes.

Individual chondrocytes decreased in volume, their cytoplasm was compacted, a significant part of the cytoplasmic processes underwent destructive changes (Fig. 7).

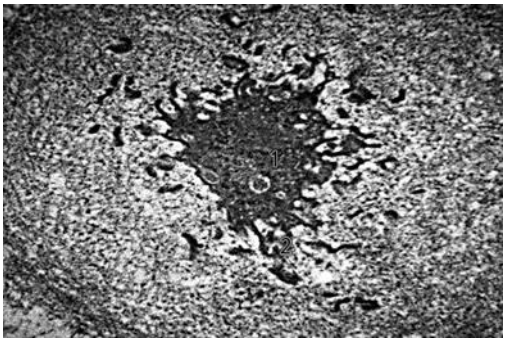

Fig. 7. Chondrocyte after 7 days of opioid exposure.
Photomicrograph. Coll. x4000.
1 - decrease in chondrocyte volume; 2 - fragmentation, destruction and exfoliation of cytoplasmic processes of chondrocytes.

In the vast majority of areas of the fibrous ring fibrils of collagen fibers were preserved. Due to the accumulation of light osmophilic mass in the interfiber proteoglycan matrix of the fibrous ring in small areas, the stratification of collagen fibers was noted. Some fibrils of collagen fibers were displaced in the area of the gelatinous nucleus. Vacuoles filled with inhomogeneous osmophilic masses accumulated in places in the stroma of the fibrous ring.

As a result of the study of the experimental material for **14 days** in the skin, which was under the influence of opioid analog analysis at a dose of 15 mg / kg at the ultrastructural level, it was found necrotic changes in cells gelatinous nucleus, as well as the development of

collagen fibers of the fibrous ring. In particular, there is the use of a heterochromatic system in the nucleus of notochondral cells, which accompanies the reduction of the nucleus due to the inhomogeneous swelling of the cytoplasm.

In chondrocytes, an increase in the destruction of cytoplasmic processes was observed, which in some cells was accompanied by moderate hyperplasia of the structural components of the Golgi complex and the granular endoplasmic reticulum. The number of vacuoles of different sizes increased in the cytoplasm of chondrocytes. Glycogen grains were not found in the cytoplasm. In some cells there was an increase in osmophilicity and compaction of the nucleus.

The content of granular intensive osmophilic inclusions increased in the matrix of the gelatinous nucleus, fibrils of slightly thickened collagen fibers were more often visualized.

Active fibroblasts were often visualized in the fibrous ring. A large part of the nucleus of such cells was filled with light euchromatin areas, and the cytoplasm was filled with organelles of protein synthesis, in particular dilated tubules of the granular endoplasmic reticulum with a significant number of ribosomes on their surface **(Figs**. **8, 9).**

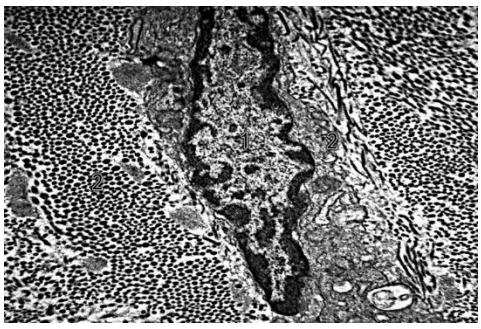

Fig. **8**. Fibrous ring after 14 days of opioid exposure. Photomicrograph. Coll. x4000.
1 - fibroblast; 2 - fibrils of collagen fibers.

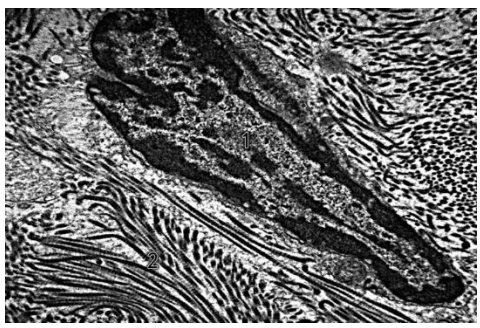

Fig. 9. Fibrous ring after 14 days of opioid exposure. Photomicrograph. Coll. x4000.
1 - fibroblast; 2 - inhomogeneous fibrils of collagen fibers.

Inhomogeneous thickening, chaotic arrangement and stratification of individual fibrils of collagen fibers were noted **(Figs. 10, 11).** The stratification of collagen fiber fibrils occurred due to the accumulation of fine-grained medium electron density in the interfiber proteoglycan matrix.

Fig. 10. Fibrous ring after 14 days of opioid exposure. Photomicrograph. Coll. x4000.
1 - fibroblast; 2 - fibrils of collagen fibers of the fibrous ring;
3 - inhomogeneous thickening and stratification of individual fibrils of collagen fibers.

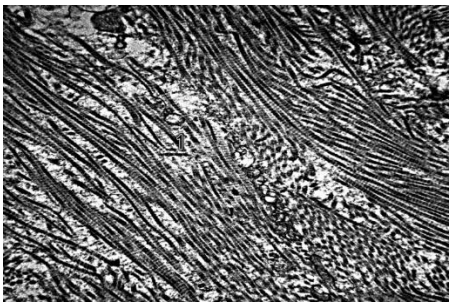

Fig. 11. Fibrous ring after 14 days of opioid exposure. Photomicrograph. Coll. x6000.
1 - inhomogeneous and chaotic stratification of fibrils of collagen fibers of the fibrous ring.

Intense osmophilicity and thickening of fibrils of collagen fibers of the fibrous ring were observed in some areas of the fibrous ring. Focal destruction of collagen fibers was registered **(Fig. 12).** In the areas of destruction, the fibrils of collagen fibers disintegrated into an inhomogeneous fine-grained mass, stratified, and were located loosely.

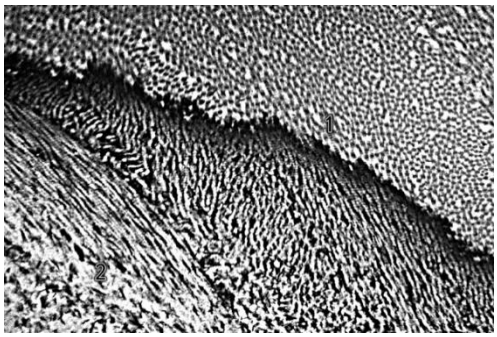

Fig. 12. Fibrous ring after 14 days of opioid exposure. Photomicrograph. Coll. x6000. 1 - inhomogeneous thickening of fibrils of collagen fibers of the fibrous ring; 2 - focal destruction of collagen fibers.

Conclusions:

1. At the end of the first week we found signs of experimental opioid effects, characterized by the development of moderate degenerative changes of individual notochondral cells, accompanied by increased vacuolation of the cytoplasm by inhomogeneous compaction of the nucleus, an increase in heterochromatin. Degenerative changes were recorded in chondrocytes, which were accompanied by the appearance of bulky vacuoles in the cytoplasm, filled with inhomogeneous osmophilic content. The cytoplasmic processes of chondrocytes decreased in volume, shortened, fragmented, and some of them detached from the surface of the plasmolemma.

2. At the end of the second week, signs of experimental opioid effects progressed, manifested by increased destruction of cytoplasmic processes in chondrocytes. In some cells, these phenomena were accompanied by moderate hyperplasia of the structural components of the Golgi complex and the granular endoplasmic reticulum. The number of vacuoles of different sizes increased in the cytoplasm of chondrocytes. Intense osmophilicity and thickening of fibrils of collagen fibers of the fibrous ring were observed in some areas of the fibrous ring. Focal destruction of collagen fibers was recorded.

Conflict of interest: None.

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