MORPHOLOGY AND PATHOMORPHOLOGY

Morphological Changes in Intervertabral Disk Tissues in a Static Asymmetrical Compression Model of Degenerative Dystrophic Diseases of Intervertabral Disks

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 146, No. 9, pp. 336-340, September, 2008
Original article submitted May 5, 2008

Morphological studies showed that the model of compression static asymmetric degenerative diseases of intervertebral disks in rats developed by us corresponds to degenerative diseases of the spine in humans. Three-month compression led to a significant reduction of the total disk height by 15.3% and a reduction in the content of notochondrial cells by 64.8%.

Key Words: *intervertabral disk; osteochondrosis; tail; rat*

The study of the processes in the intervertabral disks (ID) during their degenerative changes and development of new effective therapeutic approaches are impossible without experimental studies on laboratory animals. The main objects of the study in experimental models are cellular and chemical composition of nucleus pulposus (NP), annulus fibrosus, end plate tissues, and bone structures adjacent to the disk.

The majority of authors attribute the appearance of degenerative changes in ID tissues to reduction in the number of cells producing and regulating the synthesis of extracellular matrix under the effects of unfavorable factors, for example, bipedalism and high pressure on ID, associated with it, particularly in the lumbar compartment, exposed to the greatest load. Traumatic factors also play an important role [4].

The main structure maintaining functional characteristics of ID is hydrophilic NP providing the hinge and buffer characteristics of the joint. It is assumed that the structural and functional units of NP are cartilage-like, or notochondrial, cells, supporting the NP hydrophilia by synthesizing hydrophilic proteoglycanes (aggrecane).

It was shown that high pressure (even under culturing conditions) negatively affects viability of notochondrial cells [3]. This observation is in line with the results obtained in the analysis of autopsied ID tissues from humans of different age groups dead from conditions not related to vertebral pathologies. Compression leads to cessation of the extracellular matrix remodeling and to the appearance of signs of tissue degeneration manifesting in increased activities of metalloproteinases and especially aggrecanases (ADAMTS-4,5,7) [6]. NP cannot longer function as a "hinge" and "buffer", shrinks, and is sequestrated. Signs of necrosis and apoptosis are detected in it in equal shares. Asymmetrical pressure, particularly in compartments of physiological curvature of the spine, leads to the

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0007-4888/08/1463-0361

formation of stratification and rupture of fibers, which clinically manifests in vertebral hernias [1,10-12].

Changes in ID tissue cell composition leads to modification of their chemical composition. The main markers of degenerative diseases of the cartilaginous tissue are such processes as proteoglycane (aggrecane) destruction, collagen-2 denaturing, increase of collagen-1 percentage, manifestation of sclerotic changes, and appearance of free glycosaminoglycans (GAG). Bound and free water is redistributed inside NP and annulus fibrosus, which leads to dehydration of tissue and loss of their buffer function [8].

We studied the degenerative changes in ID of rats on our compression static model of degenerative ID diseases.

MATERIALS AND METHODS

The experimental model is based on asymmetrical static ID compression in the caudal compartment of the spine without using external or internal fixing elements. The study was carried out on male Wistar rats (n=35; 12-15 months, 450-550 g; Stolbovaya Breeding Center), in which $^2/_5$ of the caudal compartment (Cc20-21) of the spine was resected and the stump was sutured under the skin of the back 1 cm cranially from the lumbosacral articulation. For stronger fixation, armed suture was made with unresolved suturing material (prolen 5/0) between the terminal vertebra and skin of the back above and below the operation wound. The skin of the resected part of the tail was sutured to the skin of the back (vicril 5/0). This created compression of ID at the Cc5-6, Cc6-7, and Cc7-8 ID level with the maximum pressure in the Cc6-7 region, where a 20° angle between the articular surfaces of the neighboring vertebrae formed, similar to the angle in the lumbar compartment of human spine. Manipulations on experimental animals were carried out in accordance with the Order of Ministry of Health of the USSR No. 7555 of August 12, 1977. Experiments were allowed by Bioethics Committee of Institute of Human Morphology.

Intact animals (n=5) formed control group 1, sham-operated animals (n=5) subjected to resection of the caudal compartment of the spine without compression formation formed control group 2. Animals in which asymmetrical static compression of ID was formed for 1 month comprised experimental group 1 (n=5), those exposed to compression for 2 months constituted experimental group 2 (n=5), and animals exposed to ID compression during 3 months formed experimental group 3 (n=5).

Histological study of tissue samples was carried out directly after sacrifice. Intervertabral disks at Cc6-7, Cc7-8, and Cc8-9 level exposed to the highest pressure were examined. The samples were put into formalin in Lilly phosphate buffer for 72 h. Decalcination was carried out in hypersaturated EDTA solution, after which the samples were embedded in paraffin (Histomix, Biovitrum) and 7-μ sections were prepared, stained with hematoxylin and eosin, by Mallory's method, with safranine O with poststaining by light green, alcyan blue after Mowry at pH 1.0, and with gallocyanine. Morphometrical studies were carried out using Motic Images Plus 2.0 morphometrical software.

The following parameters characterizing the main components of the pathogenesis of ID degenerative changes were analyzed: ID height, area of NP section in the central part of the disk, angle between the end plates, number of nucleated cells in NP in the central part of the disk. ID height was evaluated by measuring the height of the closest to each other epiphyseal surfaces of the vertebrae above and below the measured ID in the compression region and the most distant epiphyseal surfaces of the upper and lower vertebrae in the compression region (Fig. 1). The area of NP section was measured automatically by outlining the NP contour in the preparation (Fig. 1). The angle between the end plates was measured automatically by placing the angle sides tangentially to the protruding epiphyseal parts adjacent to ID (Fig. 1). Nucleated cells on the NP section in the central part of the disk were detected by transforming color images into monochromatic ones and its inversion (Photoshop CS2). The nuclei after this procedure looked like white points against the black background. For more convenient counting, a grid (10×10 mm) was superimposed on the image. The cells were counted using a hematological counter.

The data were statistically processed using Statistica 6.0 software. As the distribution of the data differed from the normal, Kruskal—Wallis ranked dispersion analysis and nonparametric Dunn test were used for comparison of the values. The results were expressed as the mean±standard deviation at p<0.05 level of significance.

RESULTS

Normal ID in the caudal part of rat spine consists of annulus fibrosus with longitudinally oriented fibers. Solitary spindle chondrocytes are seen between the fibers. Staining with safranine O or alcyan blue after Mowry shows that the cells are surrounded by glycosaminoglycan-rich jelly extra-

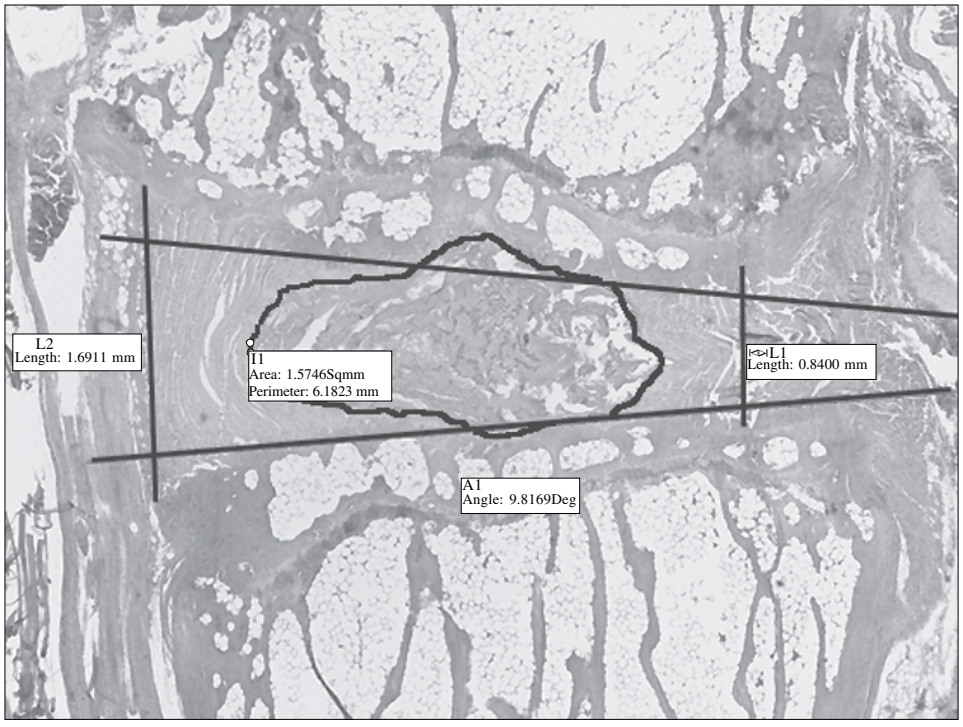

Fig. 1. Morphometric parameters of ID. Hematoxylin and eosin staining, ×5. L1: ID height in compression zone; L2: ID height in decompression zone; I1: NP area in the section; A1: angle between end plates.

cellular matrix. An oval NP is situated in the central part of ID; it occupies the greater area of the disk. Notochondrial cells form clusters of 4-8 cells, which, in turn, form accumulations of irregular shape. The extracellular matrix occupies the greater part of NP. The notochondrial cell cytoplasm is poorly stained with safranine O and alcyan blue. The end plate consists of 1-2 layers of round or spindle basophilic cells in the central part of the disk, forming bars at the site of NP fiber fixation. The tissues adjacent laterally are presented by loose fibrous connective tissues with large vessels and bundles of tendons in the tendon sheaths. The bone part of the vertebrae adjacent to ID is presented by the epiphysis and growth zone contacting with the bone marrow cavity of the vertebral body.

Histological study of the caudal ID in experimental group 1 showed well-discernible compression zone of annulus fibrosus by approximating epiphyses and the decompression zone on the contralateral side. Fibrils of annulus fibrosus in the decompression area are strained, retain their longitudinal orientation, and are well stained with safranine O and alcyan blue after Mowry. Chondrocytes are flattened on the fibers. Annulus fibrosus fibrils in the compression zone become more and more twisted, their bundles are loosened. Chondrocytes are located along the fibers. Some bundles of annulus fibrosus fibrils are directed out of, while others inside the ID, compressing and displacing the NP. The content of GAG is higher in the bundles directed to NP, but areas with high content of GAG are seen between the afferent fibers around chondrocytes. The content of GAG is not increased in the fiber compression region. NP is decentralized. The structure of the extracellular matrix is jelly

TABLE 1. Main Morphometric Parameters of the Rat ID ($M \pm m$)

Parameter	Control 1	Control 2	Experimental group 1	Experimental group 2	Experimental group 3
ID height at the site of compression, mm	2.26±0.09	2.20±0.08	2.12±0.12	1.27±0.24	0.58±0.10
ID height at the site of decompression, mm	2.29±0.07	2.24±0.08	3.37±0.36	2.84±0.42	1.92±0.36
NP section area in central part of the disk, mm^2	4.78±0.16	4.87±0.15	4.17±0.22	2.51±0.53	1.18±0.44
Angle between end plates, degrees	—	—	13.94±1.45	25.43±1.41	34.13±1.66
Cell count, count of nuclei per NP section	1060.5±88.8	1028.6±73.5	386.8±15.4	379.7±63.6	363.9±41.1

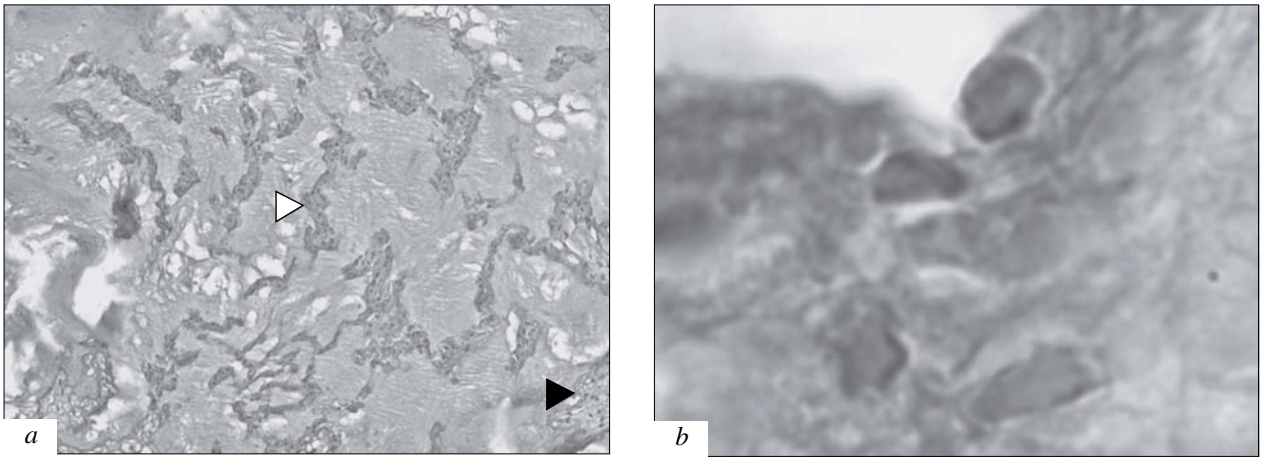

Fig. 2. Histological picture of changes in NP after 1 month of asymmetrical static compression of the caudal part of rat spine. *a*) degenerative notochondrial cells (light arrow) and unchanged cells (dark arrow); *b*) oxyphilic karyoplasm of degenerative cells. Hematoxylin and eosin staining, ×40 (*a*), ×100 (*b*).

and tends to form cords. The extracellular matrix is thickly stained with safranine O and alcyan blue after Mowry. Hematoxylin staining detects degenerative changes in large groups of notochondrial cell clusters with oxyphilic karyoplasm (Fig. 2). The bone part of the epiphyses is somewhat thickened, particularly at the site of compression, the bone bars are thick and short. Bone marrow cavities are somewhat smaller. Lesions in the growth zones are seen in some visual fields.

Morphological changes in experimental group 2 are similar to those in group 1, but annulus fibrosus damage, NP decentralization, and epiphyseal changes are more pronounced.

Morphological changes in ID progressed in experimental group 3. In some sample, the epiphyseal bone at the site of compression was thinned until complete disappearance, and areas of cartilage tissue growth were seen laterally from these sites, this cartilage tissue being replaced by bone tissue with the formation of pricky processes.

The height of the disk in the compression and decompression zones differed significantly in all groups and exhibited a trend to total reduction with prolongation of exposure. The NP section area in the central part of the disk differed significantly in all groups except control groups 1 and 2. The angle between the end plates increased significantly in all groups except control groups 1 and 2. The number of nuclear cells in NP was virtually the same in experimental groups 1, 2, and 3, but the count of these cells decreased 3-fold in comparison with control groups 1 and 2 (Table 1).

Static asymmetrical compression simulating ID compression in humans in areas of physiological spinal curvature leads to progressive reduction of the count of notochondrial cells, condensation and destruction of NP extracellular matrix, decrease of its volume and decentralization to the side contralateral to compression, and to total reduction of the disk height after longer exposure. Asymmetrical compression caused loosening and ruptures of fibers in annulus fibrosus with the formation of tissue destruction foci. The posterior part of ID is the site of this compression in the lumbar compartment of the spine, which fact explains the higher incidence of posterior spinal hernias *vs.* anterior ones, because during inclination forward the NP shifts backward and passes the zone of injury (annulus fibrosus ruptures), thus forming a hernial protrusion. However, vascular growth in ID in compression models on rodents remains unclear [5]. This observation can be explained by high concentration of antiangiogenic factors (angiostatin, hypoxia inducing factor-a, chondromodulin-1), released by annulus fibrosus fibroblasts. It seems that NP, in turn, has no sufficient concentration of these substances, which was shown in our study, as the vessels grew towards NP only through the damaged annulus fibrosus zone. Experimental models of degenerative ID diseases with primary injury to annulus fibrosus always showed vascular growth and formation of fibrous tissue, represented by collagens 1 and 3, in the center of the disk, which can be caused by low expression of antiangiogenic factors in NP. Hence, experimental morphological study of a model of degenerative ID diseases detected several major pathophysiological components.

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