Compression Asymmetric Static Experimental Model of Degenerative Disk Diseases

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We propose an experimental model of degenerative-dystrophic changes in intervertebral disks of rat tail. The results of X-ray examination and histological studies showed that degenerative changes in the disk tissues caused by experimental compression of intervertebral disks in rat tails are identical to those in humans.

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

Back pain syndrome is one of the most prevalent pathologies all over the world. It is now accepted that degenerative disease of the spine is evolutionary determined by upright posture. Traditional methods only partially alleviate some symptoms of the disease, but cannot stop pathological changes leading to degeneration of tissues in the intervertebral disks (IVD). Creation of new effective methods of treatment is impossible without experimental modeling of the disease on laboratory animals. Published data suggest that the models of IVD degeneration can be divided into two groups.

Group 1 models includes mechanical injury to IVD (disk destabilization by damaging annulus fibrosus, traumatic disruption of the nucleus pulposus with needles or its aspiration [5-8], or modeling the axial load, *i.e.* IVD compression).

Another group includes damages leading to degenerative changes in IVD tissues (treatment of the nucleus pulposus with chemical agents, *e.g.* proteolytic enzymes or apoptosis inductors) [9,10]. Chemical agents used in this model induce death of notochordal cells via induction of apoptosis or due to necrotic changes and tissue lysis.

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Degenerative disease of IVD develops spontaneously in some animal species (*e.g.*, sand rat *Psammomys obesus*). Adult animals (at the age of ~18 months) develop root syndrome and more than half animals have histological signs of degenerative changes in IVD. X-ray examination revealed spontaneous development of lumbar spondilosis [3]. However, the possibility of using these animals as a biological model of spine disease was not yet proved.

Mechanical compression seems to be an optimal model, because physiological curvatures of human spine determined by upright posture lead to asymmetric compression of IVD. The majority of experimental studies involving osteochondrosis modeling by the compression method are performed on the caudal segment of rat spine using Illizarov-type apparatus [2,4]. According to published data this model had some drawbacks: damage to the bone tissue of neighbor vertebra with pins and damage to soft tissues; potential risk of infection and development of chronic latent infectious and inflammatory process in tissues, which can affect the results of the study; reaction of tissues to foreign body; laboratory animals used in this model require special care.

The experimental compression model of degenerative IVD diseases proposed by us is free from these drawbacks.

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MATERIALS AND METHODS

Experiments were carried out on 12-15-month-old male Wistar rats weighing 450-180 g. Resection of $^2/_5$ length of the caudal segment of the spine at the level of Cc_{XX-XI} was performed and the stump was sewed under the skin on the back 1 cm cranial to the lumbosacral joint. The reinforcing sutures were made with a nonabsorbable suture material (Prolen 5/0) between the vertebra and skin above and below the operation wound. An anastomosis was applied between the skin on the back and tail (Vicril 5/0), *i.e.* compression was created at the level of Cc_{V-IV}, Cc_{VI-VII}, $Cc_{VII-VIII}$ IVD with maximum pressure at the level of Cc_{VI-VII}, where the angle between the joint surfaces of neighbor vertebra was ~40°. From resected IVD annulus fibrosus, nucleus pulposus, and endplate were isolated and transported to the laboratory in a special medium for isolation of cell cultures.

Anesthesia was performed by inhalation narcosis with diethyl ether and regional anesthesia with 0.5 ml marcaine.

X-ray examination was carried out at the Department of Neuroradiology, N. N. Burdenko Institute of Neurosurgery, Russian Academy of Medical Sciences. Narcotized animals (Zoletil-50, 5 mg/kg) were transported to the laboratory in a special semirigid polyurethane foam fixator. In most cases, first spiral computed tomography (SCT) and then magnetic resonance imaging (MRI) were carried out, after that the animals were returned to the vivarium. MRI was performed on a 1.5-T scanner (GE Fast Spin Echo XL/90) using pulse sequence with the following parameters: TR5420, TE94, 3 mm section thickness, 0.7 mm distance between sections, 320×256 matrix, FOV 20/20.

Because of low body weight of the experimental animals, a 2-liter jar with tap water was placed inside the coil near the animal, which allowed us to obtain high-quality images.

Spiral computed tomography was performed on a 6-detector spiral computed scanner Phillips (0.75 slice thickness, FOV 500, 512 matrix).

The animals were sacrificed after 3 months by diethyl ether overdose.

For histological studies, IVD samples were fixed in formalin after Lilly. Sections (10 μ) were prepared routinely and embedded in paraffin [1]. The sections were stained with hematoxylin and eosin, after Mallory, with safranine with poststaining with light green, and with alcian blue after Maury.

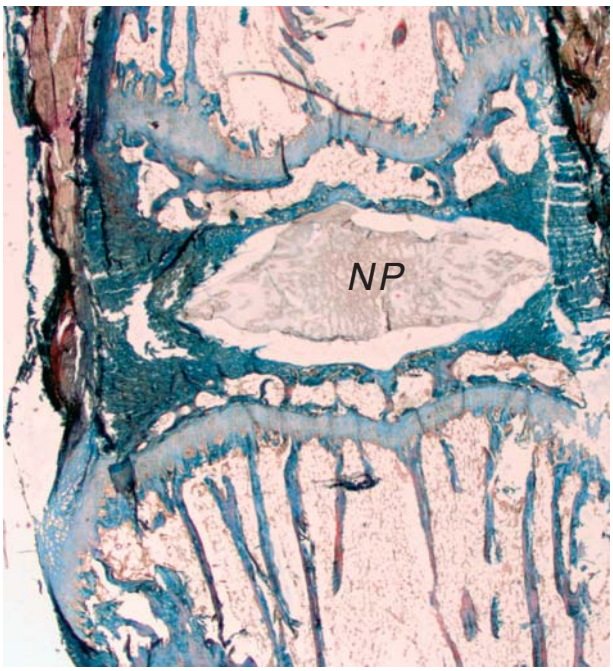

Fig. 1. Histological preparation of IVD from a 12-month-old rat: physiological norm. NP: nucleus pulposus. Longitudinal fibers: annulus fibrosus. Mallory staining, ×25.

RESULTS

Examination of normal IVD from 12-15 month-old rats at the level of Cc_{VII}-Cc_X revealed a clear-cut cartilage layer between vertebral metaphyses and epiphyses (growth zone). The vertebra were presented by compact and spongy bone containing the bone marrow. IVD was presented by nucleus pulposus, annulus fibrosis consisting of thick longitudinal collagen fibers, and narrow endplate (Fig. 1). Reaction for total glycosaminoglycans revealed their accumulation in the central zone of the annulus fibrosus adjacent to the nucleus pulposus and in the main substance of the nucleus pulposus. Notochordal cells formed clusters of 5-7 cells in the gel matrix.

Thus, the histological picture and chemical composition of IVD from the caudal segment of 12-15-month-old rats were similar to those in humans. However, the ratio of nucleus pulposus to annulus fibrosus area in rat tail is shifted towards nucleus pulposus. Considerable accumulation of the main hydrophilic components of the extracellular matrix, basic and acid glycosaminoglycans, was observed in the nucleus pulposus, annulus fibrosus, and endplate.

Progressive degenerative changes were observed in IVD tissues of the caudal spinal segment after 3-month asymmetric compression: decreased height of the nucleus pulposus and its condensation and the formation of osteophytes (Fig. 2). Histological study showed that 3-month asymmetric com-

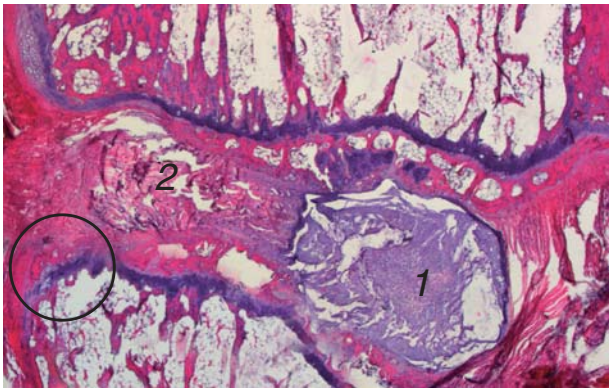

Fig. 2. Histological preparation of IVD tissues after 3-month asymmetric compression: degenerative processes. *1*) decreased height of the disk at the site of compression, decentralization of NP; *2*) partial necrosis of annulus fibrosus ay the site of maximum pressure. Circle: osteophyte formation. Hematoxylin and eosin staining, ×25.

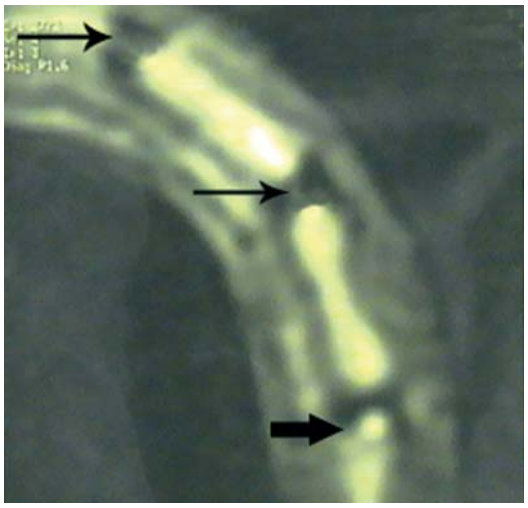

Fig. 3. Magnetic resonance imaging 1 month after surgery. T2-weighted image. Thin arrows: degenerative and dystrophic changes in IVD caused by compression; thick arrow: IVD without compression (tissue hydrophilicity is preserved).

pression of IVD led to morphological changes resembling degenerative processes in human IVD: decrease in disk height, displacement of the nucleus pulposus from the center and its dehydratation, condensation, and fragmentation, disturbances in fibrous structure of the annulus fibrosus and formation of osteophytes.

SCT performed 1 month after compression in the same rat showed asymmetric decrease in IVD height, which was most pronounced in $Cc_{V\text{-}VII}$. By their mathematical characteristics the observed changes are similar to lordosis in humans. MRI in T2 regimen performed after 1-month compression in the same animal revealed decreased signal from the nucleus pulposus. These findings resemble so-called black disk observed in patients with IVD degeneration (Fig. 3).

Thus, we developed a new experimental compression static asymmetric model of degenerative disk diseases on the caudal segment of rat spine. It was shown that the first changes in IVD appear as soon as after 1 month of asymmetric compression, which was confirmed by the results of morphological studies and X-ray examination. Histological picture of stage IV degeneration of IVD was observed after 3 months.

This experimental model can be used for studying of the pathological processes in IVD tissues and for the development of new treatment methods. Moreover, the resected distal segment of the tail can be used for isolation of cell cultures of multipotent mesenchymal bone marrow stromal cells, chondroblasts, and notochordal cells for creation of cell technologies for the treatment of degenerative disk diseases.

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