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Age features of structural rearrangements of the vertebral motor segment in conditions of limited motor activity

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Purpose: *to study in the age aspect structural changes of the lumbar intervertebral discs and adjacent structures of the spine of rats with limited motor activity to determine the patterns of their restructuring under conditions of experimental hypokinesia.*

Material & Methods: *study was performed in an experiment on male Wistar rats of different ages. The hypokinesia regimen was achieved by keeping animals in small cells, which restricted movement. The research methods included morphometry, macro- and microscopy, standard histology methods with hematoxylin-eosin and picrofuchsin staining according to Van Gieson, as well as statistical analysis of the data were obtained.*

Results: *it is determined that the restriction of mobility contributed to the change of all parts of the vertebral motor segment. The shape and linear dimensions of the intervertebral discs change, the level of its diffuse feeding from the vertebral bodies decreases.*

Conclusions: *it has been established that the absence of mechanical stimulation of intervertebral disks, in the mode of experimental hypokinesia, reduces the level of metabolism in cells, inhibits the processes of adaptive tissue remodeling and contributes to the disruption of intact structures.*

Keywords: *experimental hypokinesia, vertebral motor segment, intervertebral disk.*

Introduction

The state of insufficient motor activity of the body or hypokinesia is an extreme factor in the modern environment. The decrease in motor activity tends to spread due to the introduction of advanced technologies and widespread mechanization and automation, replacing physical labor. There is scientific evidence that in conditions of hypokinesia, which is caused by a sedentary lifestyle of a modern person, the structure of articular and metaphysical cartilages is disturbed (B. A. Nikityuk, B. I. Kogan, 1974; B. I. Kogan, S. I. Lominoga, 1978; V. G. Koveshnikov et al., 1980; I. V. Khrustaleva, B. V. Krishtoforova, 1987; L. A. Vieira et al., 2018). Regarding the intervertebral disc (ID), the complex morpho-functional assessment of its rearrangements under various conditions of hypokinesia still requires its study. The intervertebral disc is the central link in the vertebral motor segment, the lesion of which triggers the dystrophic processes of the adjacent structures of the spine and leads to the development of osteochondrosis (V. G. Kolotusha and others,2005; M. De Christopher et al., 2018; H. N. Fernando et al., 2011).

Among the diseases affecting the vertebral motor segments, degenerative diseases are much more common than neoplasms, inflammatory diseases and developmental pathologies, in connection with which degenerative diseases of the spine become of paramount clinical importance (L. A. Vieira et al., 2018; V. G. Kolotusha and others, 2005; J. Kremer 2013; Hui Li et al., 2017; Justin A. Iorio et al., 2016), including in sports (V. A. Kolesnichenko, V. A. Stroud, 2005; V. N. Levenets, 2002).

In this regard, it is important to clarify the response of the system of ID to various modes of hypokinesia.

Purpose of the study: to study in the age aspect structural changes of the lumbar intervertebral discs and adjacent structures of the spine of rats with limited motor activity to determine the patterns of their restructuring under conditions of experimental hypokinesia.

Material and Methods of the research

The study was performed on male Wistar rats of three age groups: 1, 3 and 12 months of age. Work with laboratory animals was carried out in accordance with the requirements of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes", 1986. A total of 90 animals of the experimental series (ES) and 90 animals of the control series (CS) were observed in the experiments.

The hypokinesia regimen lasted 23 hours a day and was achieved by keeping animals in small cells, which limited their movement. Feeding animals consistent with the standard diet of vivarium.

The research methods included a macroscopic assessment of the state of ID, a study of the vascular bed of the vertebral region, which is bordered by ID, with a filling of blood vessels with ink and gelatin mass, methods of histological, histoenzymological, electronic studies, morphometry, cytophotometry and statistical analysis of the data.

Macro and microscopic studies were performed using a MBR-1 binocular loupe; at the same time, the shape, color,

consistency and degree of preservation of MD were evaluated.

For histological studies, the material was fixed in 10% neutral formalin and was compacted into celloidin after conventional treatment. Sections from tissues 10–12 μm thick were stained with hematoxylin-eosin and pikrofuksin according to Van Gieson.

Histochemical analysis was performed after staging reactions on certain enzymes of the Krebs cycle and glycolysis on frozen sections. The activity of the following enzymes Krebs cycle: malate dehydrogenase (EC 1.1.1.37) (MDH), succinate dehydrogenase (EC 1.3.99.1) (LDH) of glycolytic enzymes – alpha-glycerophosphate dehydrogenase (EC 1.1.1.8) (α-GPDH) and lactate dehydrogenase (EC 1.1.1.27) (LDH) and its isoenzymes.

Enzyme activity was assessed on a two-beam scanning cytospectrophotometer MUF-5. Measurements were carried out using the plug-in method with a working wavelength of 546 nm, a probe diameter of 200 μm and a lens 50 Preliminary study was made of the suitability of the material in photometric works by recording the absorption spectrum on objects of various densities.

Electron-microscopic studies were carried out on an electron microscope EMS-100 BR with an increase from 12,000 to 30,000.

After applying the prefix (2,5% glutaraldehyde) and saline, the material was fixed in osmionic acid, dehydrated and compacted in an organic resin – dupupan.

To assess the conditions of diffuse nutrition of the ID, the blood supply to the subchondral parts of the vertebral bodies was studied, with the adjoining ID. The filling of the vascular bed was carried out with a 5% solution of ink with gelatin with the application of heparin. The number of microvessels in the cranial and caudally located vertebrae, corresponding to the four zones of the vertebral motor segment, was calculated on enlightened sections with a thickness of 20 μm:

Zone 1 – ventral part of the body of the cranially located vertebra
Zone 2 – the dorsal part of the body of the cranially located vertebra
Zone 3 – ventral part of the body of the caudally located vertebra
Zone 4 – dorsal part of the body of the caudally located vertebra.

Micromorphometry included measurements of the cranio-caudal and ventro-dorsal diameters of the lumbar intervertebral disks with the help of an ocular screw micrometer MOS-1-15 with the calculation of the relative sizes of these diameters.

Evaluation of statistical differences was determined using Student's criterion.

Results of the research

1. Rearrangements of macro- and microstructures of intervertebral disks under hypokinesia.

As the results of the research show, the restriction of the mobility of animals leads to a change in the structure of ID. These changes took place against the background of a significant decrease in body weight of rats ($p<0,005$). After 30 weeks of hypokinesia, in ES 1-month-old rats, body weight decreased by 38,02% relative to CS, by 27,06% in 3-month-old and by 22,18% in 12-month-old (Table 1).

Under conditions of prolonged 30-day hypokinesia, the ID lost its characteristic shape, color, and consistency. They differed from the CS white and gray color and pastoznost. The form of ID gradually changed: on the sagittal sections, the disc lost a slightly wedge-shaped form and became more rectangular.

After a 30-day hypokinesia, an increase in the cranio-caudal size of the ID and a decrease in ventro-dorsal .

The increase in cranio-caudal size compared with CS was 33,22% in 1-month-old rats, 9,42% in 3-month-old rats and 12,22% in 12-month-old rats, the decrease in ventro-dorsal size was 8,59%, 15,27% and 4,14%. As a result, the relative magnitudes of the two linear dimensions of the disk increased; that in 1-month-old rats 1,27, in 3-month-olds – 1,45, in 12-month-olds – 1,93. The greatest changes in the linear dimensions and structure of the ID were observed in the V-VI lumbar segments (Fig. 1).

Counting the number of injected mascara microvessels in the subchondral parts of the adjacent vertebral bodies showed that under conditions of hypokinesia their number decreases and, as a result, the conditions of diffuse nutrition of the MD deteriorate. The greatest decrease in the number of microve-

Table 1

Changes in the linear dimensions of the fifth intervertebral disk of rats under conditions of experimental hypokinesia in the age aspect

Experiment series	Intervertebral disc size				Evaluation of statistical significance	
	Cranio-caudal size (μm)		Ventro-dorsal size (μm)			
	CS	ES	CS	ES		
	$\bar{X}_1 \pm m_1$	$\bar{X}_2 \pm m_2$	$\bar{X}_3 \pm m_3$	$\bar{X}_4 \pm m_4$	t	p
1+30	581,0±17,9	870,0±14,61	1217,0±11,07	1112,4±24.0	$t_{1,2}=12,51$ $t_{3,4}=3,96$	$p_{1,2}<0,001$ $p_{3,4}<0,001$
3+30	863,7±6,82	953,5±10,15	1640,9±17,06	1390,3±16,69	$t_{1,2}=7,34$ $t_{3,4}=10,5$	$p_{1,2}< 0,001$ $p_{3,4}< 0,001$
12+30	1175,0±5,99	1338,5±12,0	2701,1±17,95	2589,2±21,49	$t_{1,2}=12,19$ $t_{3,4}=3,99$	$p_{1,2}<0,001$ $p_{3,4}<0,001$

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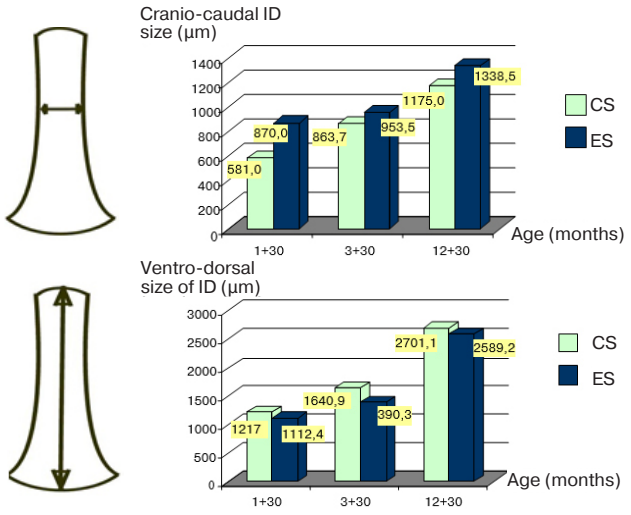

Figure. 1. Relative changes in the linear dimensions of ID after 30-day hypokinesia

ssels was found in the caudally located vertebrae and, especially, in their dorsal section (Table 2).

Hypokinesia caused the expansion of perivascular spaces and the phenomenon of perivascular edema. Microscopic experiments also found that the restriction of mobility contributes to the edema of the tissues of ID and the violation of histotopographic ratios of its components. This was manifested by an increase, almost twofold, in the volume of the gelatinous nucleus and the difference in the plates of the fibrous ring.

The gelatinous nucleus occupied a more central position in ID, structural disorders of its notochordal cells were observed. In both layers of the fibrous ring, the phenomena of dystrophy spread, the presence of cells with pyknotic modified nuclei increased. These changes were mainly noted in V-VI lumbar intervertebral discs.

2. Ultrastructural age features of intervertebral discs under hypokinesia.

After a 7-day hypokinesia, the morphological evidence of the adaptation process to the stress factor is associated with signs of dystrophic damage. Characterized by an increase in the nucleus of cells, the clearing of the mitochondrial matrix, the uneven expansion of individual tubules of the endoplasmic reticulum and the Golgi complex.

The 30-day hypokinesia contributed to damage to the cells and matrix of the fibrous ring, which was most pronounced in young animals. It was found that in these conditions in the 1-month rats the shape of the chondrocytes nucleus changed, cells with huge nuclei of unusual form appeared, and the presence of heterochromatin in the peripheral-membrane localization increased. In these cells there was a significant vacuolation of the cytoplasm, high osmophilia of its sites and the phenomena of edema organoids. Individually preserved mitochondria had a sharply enlightened matrix and several reduced chests. Permanent finds were fragments of a nuclear substance, freely arranged in the matrix.

Morphological indicators of damage to the matrix under the influence of hypokinesia were the loss of bonds of collagen fibers in bundles, the violation of the periodicity of the structure of collagen, the appearance of common and elegant sections along the fibers, as well as the fragmentation of some fibers.

ID 12-month-old rats, according to electronic studies, were more stable in conditions of even prolonged hypokinesia. However, in comparison with the control, in the collage-fiber cartilage the processes of cell and matrix dystrophy spread and the phenomena of tissue destruction developed.

3. Histochemical age-related features of intervertebral discs under hypokinesia.

Histochemical experiments proved that the detected structural changes unfolded against the background of a decrease in the activity of oxidative phosphorylation and glycolysis enzymes.

In 1-month-old rats after 30-dobio hypokinesia, the activity of malate dehydrogenase (MDH) decreased relative to the control by 13,4% with a slight change in the activity of α-GPDH. The activity of lactate dehydrogenase (LDH) decreased the most – by 60,9% and its LDH-1 isoform – by 81,5%.

In 3-month-old rats, MDH activity decreased by 10,47%, α-GPDH – by 27,08%, LDH – by 27,08%, LDH-1 – by 74,96%.

For 12-month-old rats, the activity of MDH decreased only by 3,05%, α-GPDH – by 20,02%, LDH – by 19,94%, LDH-1 by 47,94%. The most resistant to changes in all age groups was the LDH-4 isoenzyme.

Thus, adaptation to hypokinesia in young and mature animals has a certain difference, but in general, 1 month old rats were more sensitive to real estate, which is consistent with the data of microscopic studies.

Table 2

Age-related changes in the density of microvessel distribution, which were contrasted with mascara, in the lumbar vertebrae under hypokinesia ($X+S_x$)

Experimental series	Zones in the vertebral bodies			
	1	2	3	4
1 month + 7 days of hypokinesia	18,66±1,054	17,66±1,358	18,83±1,352	16,5±1,50
1 month + 30 days of hypokinesia	10,0 ±1,032	8,0 ±0,68	9,0 ±1,032	7,16±0,75
3 months + 7 days of hypokinesia	17,83±1,01	16,33±0,714	16.06±0,666	13,83±1,01
3 months + 30 days of hypokinesia	9,0±0,966	8,83±0,601	9,83±0,601	7,17±1,79
12 months 7 days hypokinesia	7,17±0,477	6,83±0,723	6,83±0,601	5,83±2,50
12 months 30 days hypokinesia	5,33±0,843	3,66±0,334	5,0±0,774	4,5 ±0,39

Conclusions / Discussion

The morphology of ID and its adjacent structures in conditions of hypokinesia is devoted to a small number of works that still lack comprehensive data on changes in all components of MD in conditions of reduced mobility (T. A. Glushko et al., 1987; V. G. Koveshnikov, A. E. Sac, 2005; A. E. Sac, 2010).

As evidenced by the data presented in the work, under conditions of hypokinesia, the communication system of the vertebral bodies of the ID undergoes significant structural-metabolic rearrangements, it may be associated with the processes of tissue edema and violation of the conditions of its diffuse nutrition.

The restriction of mobility contributes to the change of all departments of ID. The shape and linear dimensions of ID change, the level of its diffuse feeding on the part of the vertebral bodies decreases, the activity of metabolism in cells changes. These changes manifested themselves after 7 days of hypokinesia and were most obvious after 30 days of hypokinesia.

Under conditions of hypokinesia, the cranio-caudal diameter of the ID increased, while in young animals the ID lost its sphenoid shape. The gelatinous nucleus occupied a central position in ID and assumed a spherical shape. With an increase in the duration of the real estate of rats in the fibrous ring, the metabolic resources of the cells decreased and the dystrophic damages of organoids and matrix grew. These changes predominated in the fifth and sixth lumbar ID, which may be due to the difference in the blood supply of the cranial and caudal parts of the lumbar spine. In addition, during hypokinesia, the delayed processes of ossification of cartilage apophyses in the vertebral bodies were detected, which also changed the course of age-related alterations in ID and spinal segments as a whole.

The complex of data obtained suggests that the limitation of motor activity is a significant factor damaging ID. The main reason for this is the absence in the conditions of hypokinesia of the load necessary for the formation of the supporting structures of the ID. The absence of mechanical stimulation of ID reduces the level of metabolism in cells, inhibits the processes of adaptive tissue remodeling and contributes to the violation of intact structures. Prolonged hypokinesia contributes to the greatest damage to the structures of MD in young animals.

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References

1. Glushko, T.A., Gusakova, V.A. & Malova, N.T. (1987), "Changes in disc tissues after hypokinesia in rats of different ages", *Arkhiv anatomii*, Vol. 43, No. 1, pp. 50-55. (in Russ.)
2. Koveshnikov, V.G., Fedonyuk, Ya.I. & Ukrainskiy, A.V. etc. (1980), "The Effect of Hypokinesia on the Growth and Structure of Long Tubular Bones of Animals Adapted to Exercise", *Mater. I sezda anatomov, gistologov, embriologov Ukrainy*, Vinnitsa, pp. 93. (in Russ.)
3. Kogan, B.I. & Lominoga, S.I. (1978), "Morphology of the skeleton of inbred rats during hypokinesia and its rehabilitation options", *Byulleten eksperimentalnoy biologii i meditsiny*, Vol. 86, No. 7, pp. 99-101. (in Russ.)
4. Koveshnikov, V.G. & Sak, A.Ye. (2005), "Ultrastructural changes in the cells and the intercellular matrix of MD in different modes of hypokinesia in the age aspect", *Ukraïnskiy morfologichniy almanakh*, Vol. 3, No. 4, pp. 108-112. (in Russ.)
5. Kolesnichenko, V.A. & Straude, V.A. (2005). "Vertebral aspects of athletic selection and orientation of athletes", *Sportivna meditsina*, Vol. 1, pp. 171-174. (in Russ.)
6. Kolotusha, V.G., Rudenko, A.Ye. & Kadyrova, L.A. (2005), *Osteokhondroz poyasnichnogo otdela pozvonochnika* [Osteochondrosis of the lumbar spine], Kiev. (in Russ.)
7. Kremer, Yu. (2013), *Zabolevaniya mezhpozvonkovykh diskov, Medpress-inform* [Intervertebral disc diseases], Moscow. (in Russ.)
8. Levenets, V.N. (2002), "Sports Injuries - Diagnosis, Clinic and Treatment", *Mater. I Vseukr. z'izdu fakhivtsiv iz sportivnoï meditsini i LFK z mizhnarodnoyu uchastyu*, Odesa, pp. 32-35. (in Russ.)
9. Nikityuk, B.A. & Kogan, B.I. (1974), "Genotypic features of the formation of the skeleton of the hind limb in inbred animals with hypo-, normo- and hyperdynamic", *Ortopediya, travmatologiya*, No. 12, pp. 27-30. (in Russ.)
10. Radchenko, V.O., Petrenko, I.V., Golubeva, L.M. & Bengus, D.Ye. (2008), "Actual problems of arthrology and vertebrology", *Mater. mezhdunar. konferentsii, posvyashchennoy 100-letiyu so dnya osnovaniya Gosudarstvennogo uchrezhdeniya "Institut patologii pozvonochnika i sustavov im. prof. M.I. Sitenko AMN Ukrainy. Ortopediya, travmatologiya i protezirovanie*, No. 1, pp. 111-117. (in Russ.)
11. Sak, A.Ye. (2010), "Changes in the blood supply to the ventral spine under conditions of experimental hypokinesia", *Ukraïnskiy morfologichniy almanakh*, Vol. 8, No. 2, pp. 182-184. (in Russ.)
12. Khrustaleva, I.V. & Krishtoforova, B.V. (1987), "Age-related changes of articular and metaphyseal cartilage under hypodynamic conditions", *Mater. simpoziuma ESOA "Destruktsiya sustavov"*, Sochi, pp. 69. (in Russ.)
13. Christopher M.De GeerDC, MSc. (2018), "Intervertebral Disk Nutrients and Transport Mechanisms in Relation to Disk Degeneration: A Narrative Literature Review", *Journal of Chiropractic Medicine*, No. 17(2), pp. 97-105.
14. Council of Europe (1986), *European convention for the protection of vertebral animals used for experimental and other scientific purpose*, 18.03.1986, Strasbourg.
15. Fernando, H.N., Czamanski, J., Yuan, T.Y., Gu, W., Salahadin, A. & Huang, C.Y. (2011), "Mechanical loading affects the energy metabolism of intervertebral disc cells", *Journal of Orthopaedic Research*, No. 29(11), pp. 1634-1641.
16. Hui Li, Jia-zhi Yan, Yong-jie Chen, Wei-bo Kang, Jia-xi Huang (2017), "Non-invasive quantification of age-related changes in the vertebral endplate in rats using in vivo DCE-MRI", *Journal of Orthopedic Surgery and Research*, No. 12(1), pp.169-173
17. Justin A. Iorio, Andre M. Jakoi & Anuj Singl (2016), "Biomechanics of Degenerative Spinal Disorders", *Asian Spine Journal*, No. 10(2), pp. 377-384.
18. Vieira, L.A., Dos Santos, A.A., Peluso, C., Barbosa, C.P., Bianco, B., Rodrigues, L.M.R. (2018), "Influence of lifestyle characteristics and VDR polymorphisms as risk factors for intervertebral disc degeneration: a case-control study", *European Journal of Medical Research*, No. 23(1), pp. 11.
19. Zhu, Q., Jackson, A.R. & Gu, W.Y. (2012), "Cell viability in intervertebral disc under various nutritional and dynamic loading conditions: 3d finite element analysis", *Journal of Biomechanics*, No. 45(16), pp. 2769-2777.

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