ISSN 0030-5987. Orthopaedics, traumatology and prosthetics. 2025. № 3

УДК 617.547:616.711-002-003.8]:616.8-008.6]-092.9](045)

DOI: http://dx.doi.org/10.15674/0030-59872025337-46

Histological changes in adjacent intervertebral discs in a rat model of nucleus pulposus injury and spinal nerve compression

O. O. Barkov, V. Ye. Maltseva, O. A. Nikolchenko, Z. M. Danyshchuk, K. M. Samoylova

Sytenko Institute of Spine and Joint Pathology National Academy of Medical Sciences of Ukraine, Kharkiv

The prevalence of decompression and stabilization in the thoracic and lumbar spine (LS) is constantly increasing due to the high incidence of degenerative spine diseases among the working-age population. Aim: To analyze histological changes in rats in which the condition of LS was experimentally reproduced as in patients with spinal nerve compression before and after performing decompression and stabilization of the spine. Methods. The study was conducted using 19 white rats, in which the nucleus pulposus in the intervertebral disc was destroyed at the L_V–L_{VI} level. In Model 1, at the level of the damaged disc, the right spinal nerve was ligated with two double knots of suture material. In Model 2, the L_V and L_{VI} vertebral bodies were bilaterally fixed with two metal staples, pressing them on the root area. Results. No signs of inflammation were detected in any of the rats. Degenerative changes were recorded in the adjacent cranial and caudal intervertebral discs, but there was no significant difference in the scores between the adjacent intervertebral discs, both in the case of a general assessment and separately for the annulus fibrosus and the nucleus pulposus. The values obtained were ≈ 6.5 and ≈ 5 points, respectively, for the model. The sum of the scores for the annulus fibrosus of the degenerative disc of Model 1 was significantly higher compared to both adjacent discs ($p = 0.016$; $p = 0.026$), compared to the corresponding values for the cranial or caudal disc of Model 2, no difference was detected. In both models, Wallerian degeneration was detected in the more distal areas of the nerves. Conclusions: Degenerative changes occur in adjacent discs in a rat model of spinal nerve compression and intervertebral disc nucleus pulposus injury. This provides an opportunity to use this model to study treatments for disc degeneration in patients with neurological complications after spinal decompression and stabilization.

Поширення декомпресійно-стабілізуючих втручань на грудному та поперековому відділах хребта (ПВХ) постійно зростає через високу частоту дегенеративних захворювань цієї локалізації серед працездатного населення. Мета. Проаналізувати гістологічні зміни в щурів, яким експериментально відтворили стан ПВХ як у пацієнтів із компресією спинномозкових нервів до та внаслідок виконання декомпресійно-стабілізуючих втручань. Методи. Дослідження проведене з використанням 19 білих щурів, яким руйнували драглисте ядро в міжхребцевому диску на рівні L_V–L_{VI}. Модель 1 — на рівні ушкодженого диска перев'язували правобічний спинномозковий нерв двома подвійними вузлами шовного матеріалу. У Моделі 2 тіла хребців L_V та L_{VI} білатерально фіксували двома металевими скобами, притискаючи ними ділянку корінців. Результати. Ознак запалення не виявлено в жодного з щурів. Зафіксовано дегенеративні зміни в суміжних краніальному та каудальному дисках, проте за оцінкою в балах не було значущої різниці між суміжними міжхребцевими дисками, як у разі загальної оцінки, так і окремо для волокнистого кільця та драглистого ядра. Отримані значення дорівнювали ≈ 6,5 та ≈ 5 балам відповідно моделі. Сума балів для волокнистого кільця ушкодженого диска Моделі 1 була значуще більшою порівняно з обома суміжними дисками ($p = 0,016$; $p = 0,026$), порівняно з відповідними значеннями краніального або каудального диска Моделі 2, різниці не виявлено. У обох моделях зафіксовано у дистальніше розташованих ділянках нервів Валлерову дегенерацію. Висновки. У моделі щурів із компресією спинномозкового нерва й ушкодженням драглистого ядра міжхребцевого диска ПВХ виникають дегенеративні зміни в суміжних дисках, що дає змогу використовувати їх для дослідження методів лікування дегенерації дисків у пацієнтів із неврологічними ускладненнями після декомпресійно-стабілізуючих втручань на хребті. Ключові слова. Дегенерація міжхребцевого диска, щур, компресія спинномозкового нерва, дегенеративні захворювання поперекового відділу хребта, захворювання суміжних сегментів.

Keywords. Iintervertebral disc degeneration, rat, spinal nerve compression, degenerative lumbar disease, adjacent segment disease

Introduction

The issue of preventing and managing complications arising from decompression-stabilization procedures in the thoracic and lumbar spine remains an ongoing challenge. Transpedicular stabilization of the spine is one of the standard methods of surgical treatment of degenerative diseases of the spine. At the same time, incorrect placement of transpedicular screws is associated with the risk of neurological complications [1]. Neuropathic pain is one of them and is accompanied by a deterioration in the quality of life [2]. In patients presenting with neurological complications resulting from lumbar spine (LS) nerve compression, decompression-stabilization interventions (DSI) may not consistently result in complete recovery for all individuals [3]. Pre-existing neuropathic pain increases the risk of prolonged pain after spinal fusion [4]. In addition, it is important to study the condition of the intervertebral discs adjacent to the degenerative segment due to the corresponding complication that occurs after spinal fusion of the LS in 5–30 % of patients [5]. The solution to this problem involves creating new ways to treat and prevent these complications. That is, it is important to create models that will allow studying the development of changes in the spine in patients with nerve compression in the LS both before and after DSI. This will help to plan the further use of various conservative treatment methods, in particular biological therapy for adjacent segments. Animals, especially small ones, such as mice or rats, are one of the common options for modeling degenerative diseases of the spine [6–11]. S. Shehab et al. found in rats with unilateral ligation of the spinal nerve at the level of L_V that the effect of capsaicin or resiniferatoxin on the adjacent nerves L_{III} and LBI reduced pain and structural changes in the nerves [12]. This provides a direction in the creation of animal models for studying the development of structural changes in the spine similar to those in patients with degenerative spinal diseases and neuropathic pain at the affected and adjacent levels.

Objective: To evaluate histological alterations in rats following the experimental replication of lumbar spine conditions analogous to spinal nerve compression in humans, both prior to and subsequent to decompression-stabilization procedures.

Material and methods

The experimental study was reviewed and approved by the Bioethics and Deontology Committee of the State Institution "Professor M. I. Sytenko Institute of Spine and Joint Pathology of the National Academy of Medical Sciences of Ukraine" (protocols No. 215 dated 19.04.2021, No. 239 dated 18.12.2023). The research was carried out in accordance with regulatory and legislative requirements [13, 14].

Animals

The experiment was performed using 19 nonlinear white rats aged 9–10 months (body weight 430–675 g) from the population of the Experimental Biological Clinic of the State Institution "Professor M. I. Sytenko Institute of Spine and Joint Pathology of the National Academy of Medical Sciences of Ukraine"

Surgical interventions

For anesthesia, rats were injected intramuscularly with medetomidine hydrochloride (0.02 mg/kg) and ketamine (50 mg/kg). The animals were fixed in a supine position. After laparotomy, the abdominal organs were taken out, wrapped in a sterile gauze napkin and irrigated with saline. To access the ventral part of the LS, soft tissues and blood vessels (spiral-lumbar and posterior vena cava) were carefully retracted, protected with a sterile gauze napkin and retractors were installed. In the intervertebral disc at the level of segments L_V–L_{VI}, a standard perforated defect was created with the destruction of the nucleus pulposis (depth 1.5 mm) using a dental bur with a diameter of 1.5 mm.

The animals were divided into groups depending on the pattern of spinal nerve compression:

– *Model 1 (n = 9).* Between the right-sided muscles next to the damaged intervertebral disc, the adjacent spinal nerve (ventral branch of the lumbar nerves, which passes into the sciatic nerve) was visualized and separated with control of the manifestation of the motor reflex in the right pelvic limb of the rat. Then, the spinal nerve was ligated with 2 double knots of non-absorbable suture material (Fig. 1, a);

– *Model 2 (n = 10).* For spinal fusion, two stainless steel staples (medical stapler Manipler® AZ-35W, 6.9 mm × 4.2 mm, grade 316L) were used after shortening their arms to 2 mm. In the adjacent vertebral bodies of the L_V and L_{VI}, four standard perforated defects with a depth of 2 mm were created using a dental bur with a diameter of 1.2 mm bilaterally, two in the caudal part of the L_V and two in the cranial L_{VI}. Then, using the press-fit technique, metal staples were implanted into the defects, pressing the adjacent spinal nerves with them (Fig. 1, b).

Upon completion of the surgical intervention, the abdominal wall and skin wound of all rats were sutured in layers with absorbable suture material and treated with a povidone-iodine solution (10 %).

In Model 1, 2 rats died 5 and 9 days after the intervention. In Model 2, 2 individuals died during

the operation, another 2 a day later, and another 2 animals after 4 days.

Eight weeks after surgery, 11 rats were removed from the experiment by decapitation, which was due to the need to collect blood for biochemical analysis. Fragments of the L_I–S_I LS were removed for further histological examination.

Histological analysis

For histological analysis, the selected fragments of the lumbar spine L_I–S_I were fixed in 10 % neutral formalin for 5 days. After decalcification in 10 % formic acid solution, 3 fragments with intervertebral discs were cut out: cranial (L_{IV}–L_V), level of injury (L_{IV}–L_V), caudal (L_{VI}–S_I), cutting along the vertebral bodies. After that, the samples were dehydrated in ethyl and isopropyl alcohols of increasing concentration, soaked in a mixture of isopropyl alcohol and paraffin, and embedded in paraffin. Using a sledge microtome, histological sections of 6 μm thickness were made in the coronal plane, which were analyzed under a BX63 light microscope. Digital images were obtained using a DP73 camera.

The assessment of degenerative changes in three intervertebral discs for each rat was performed using the scale developed by A. Lai et al. [15]. According to it, the maximum manifestation of degenerative changes is 16 points, and the normal structure is 0 (Table 1). The assessment was not performed at the level of damage to the nucleus pulposus due to its absence.

Statistical analysis

The obtained indicators are presented as mean and standard deviation. Their comparison within one model was performed using the Wilcoxon test for related samples, and for comparison between models, the Mann-Whitney U-test was used. The difference was considered significant in $p < 0.05$. The analysis was performed using the SPSS Statistics 23 software.

Results

Model 1 (n = 7)

Cranial segment. In the intervertebral disc of the spinal segment located cranially from the damaged disc, in the outer section of the annulus fibrosus, collagen fibers were appropriately organized, only one rat (14 %) was found to have a violation. The cellular composition in the outer section consisted of fibrochondrocytes, and in the inner section of chondrocytes. The cell density was from medium to high. However, in the outer section, interfibrous ruptures/cracks in the laminae were detected in half of the rats, while in the others, there were no structural changes (Fig. 2, i).

In the nucleus pulposus, the matrix was heterogeneous in color in all animals except one. In 70 % of cases, notochondral cells were detected in the nucleus pulposus, while in the others, only chondrocytes were detected, and in one rat, degenerative cells together with chondrocytes (Fig. 2, d). In rats in which notochondral cells were detected, chondrocytes were also recorded. Among the signs of nucleus pulposus degeneration, the following were detected: cell loss in 42% of cases, accumulation of collagen fibers in 57 %, and calcification in 1 (14 %) (Fig. 2, d).

The endplates were without structural abnormalities, except for one rat, in which they were calcified, which was combined with vascular growth. At this level of the spinal segment, no signs of inflammation were detected.

Level of damage. At the level of injury to the intervertebral disc by a dental bur in the fibrous ring, all rats had impaired organization of collagen fibers, in 28 % they were disconnected and torn. The outer and inner sections did not have a pronounced difference in structure, and among the cells, only chondrocytes were determined in half of the rats, in the others both fibrochondrocytes and chondrocytes (Fig. 2, b, i). The cell density was from medium to low, in one animal it was high. Among the preserved plates of the outer section of the fibrous ring, interfibrous gaps were determined in 71 % of cases, which were combined in 28 % with fragmentation of the plates, and in 28 % the presence of detritus was detected.

Nucleus pulposis did not have a characteristic structural structure due to boron damage and was

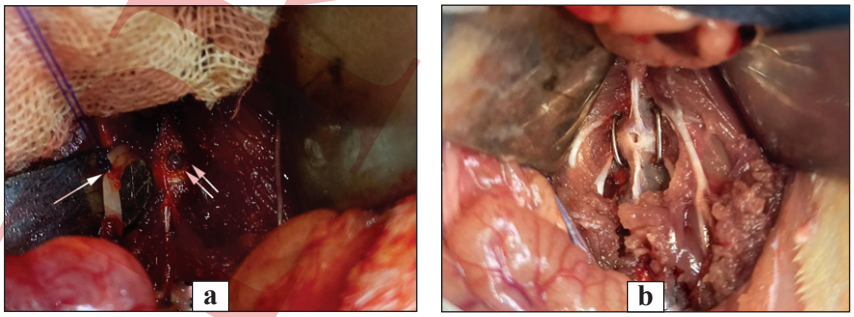

Fig. 1. Surgical site after performing a hole defect in the intervertebral disc at the level of segments L_V and L_{VI} (double arrow) and compression of the adjacent spinal nerve by ligating it with suture material in rats, Model 1 (a) and pressing with implanted metal staples in rats, Model 2 (b)

actually replaced by other tissues: in 57 % connective, sometimes with capillary-type vessels, with a large number of fibroblasts, single chondrocytes, and in 33 % cartilaginous with chondrocytes and small cells of notochondral cells (Fig. 2, f). In rats with replacement of the nucleus pulposis with cartilage tissue, cracks in the matrix and detritus were found next to them. In one animal out of 7 (14 %) the cartilage tissue in the nucleus pulposis area was combined with newly formed bone tissue cells, capillary-type vessels and detritus. There were no signs of inflammation at this level of the spinal segment.

Caudal segment. In the annulus fibrosus of the intervertebral disc of the spinal segment caudal to the injured level, 57 % of the collagen fibers retained a lamellar structure, while the rest showed signs of disorganization. The cells analyzed were characteristic of the annulus fibrosus — fibrochondrocytes and chondrocytes, which were located near the nucleus pulposus. The cell density was medium to high. Interfibrous gaps in the annulus fibrosus plates were found in half of the rats, while the integrity of the structure was preserved in the others (Fig. 2, o).

In the nucleus pulposus, notochondral cells were identified in only half of the rats, while only chondrocytes were found in the others. Notochondral cells were often combined with chondrocytes. The matrix of the nucleus pulposus was mostly heterogeneously stained. Among the signs of degeneration of the nucleus pulposus, an accumulation of collagen fibers was found in 28 %, while areas of calcification were present in the others (Fig. 2, g).

The endplates were intact with no signs of vascular ingrowth. Inflammation was not detected in any of the studied cases.

At this level, the spinal nerves that formed the lumbar plexus were surrounded by an epineurium of dense connective tissue. (Fig. 3, a, b). In the nerve fibers, degenerative changes in axons were determined in the form of accumulation of amorphous decay products and axon fragmentation (Fig. 3, a). Single Schwann cells were determined (Fig. 3, b).

According to the quantitative assessment of degenerative changes in intervertebral discs in Model 1, no difference was found in the scores between the cranial and caudal adjacent discs (Table 1), and the average score was ≈ 6.5 points. The sum of the scores for the fibrous ring of the damaged disc was significantly higher compared to both adjacent discs ($p = 0.016$; $p = 0.026$).

Model 2 (n = 4).

Cranial segment. The intervertebral disc located cranially from the damaged segment consisted of a fibrous ring and a nucleus pulposis (Fig. 2, a). In the fibrous ring of the intervertebral disc, collagen fibers retained the appropriate structural organization in 80% of rats. Fibrochondrocytes were located in the outer and chondrocytes in the inner part of the fibrous ring. The cell density was average. In the outer part of the fibrous ring, interfibrous gaps and fiber breaks in the laminae were found in 40 % of cases (Fig. 2, p, w).

In the nucleus pulposis, the matrix had a heterogeneous color in 60 % of rats, the cellular composition in all animals was notochordal, and chondrocytes were also found in 40 % of them (Fig. 2, n). Among the degenerative signs, the accumulation of collagen fibers in areas was determined in 60 % of cases. The endplates were calcified in 80 % of the rats, but without vascular growth. No signs of inflammation were found in any animal.

Level of injury. At the level of injury, signs of disorganization of collagen fibers in the annulus fibrosus of the intervertebral disc were determined in 80 % of the individuals (Fig. 2, q, x). Chondrocytes and fibroblasts predominated among the cells. Their density was high in 60 %, and medium or low in the others. Interfibrous gaps in the laminae of the annulus fibrosus and loss of the lamellar structure were also found in 80 %.

The nucleus pulposus did not have a characteristic structure and was replaced in 80 % by cartilage tissue (Fig. 2, r, u, Fig. 4, a), one of these rats was found to have connective and bone tissue simultaneously (Fig. 4, b).

In one animal (20 %), only connective tissue was identified in the area of the nucleus pulposus. In a rat with bone tissue, capillary-type vessels were found in the connective tissue. The cells were appropriate for the type of tissue, fibroblasts in the connective tissue, chondrocytes in the cartilage tissue, osteoblasts and osteocytes in the bone tissue. The endplates were calcified in 60 % of the animals, and in the others they were completely absent. No signs of inflammation were detected.

In the vertebral bodies adjacent to the intervertebral disc, holes were identified that remained from the staples installed during the surgical intervention to stabilize this spinal segment. Bone tissue of a lamellar structure was formed around the holes. Cartilage tissue was also identified along the perimeter of the staples, and connective tissue in small areas (Fig. 5, a). The structure of the vertebral bodies was without signs of disorders. The only peculiarity was that newly formed connective tissue with capillary-type vessels was found on the outside of the vertebral

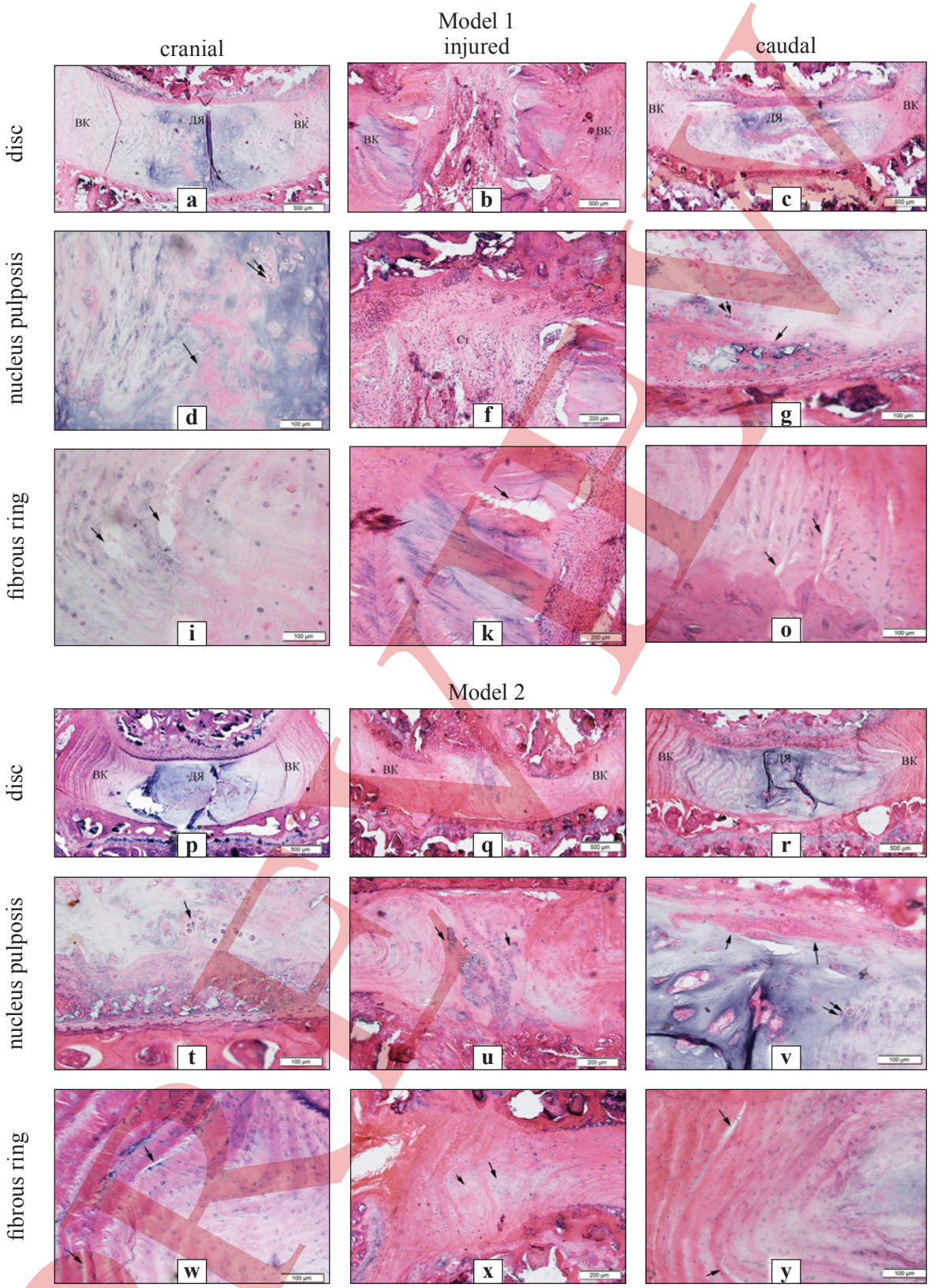

Fig. 2. Structural features of intervertebral discs in Models 1 and 2 at the level of injury and adjacent (cranially and caudally) to this spinal segment. General view of the structure of the intervertebral disc (a–c, p–r). Replacement of the nucleus pulposis with connective tissue (Ct) in Model 1 (b, f) or with cartilage tissue in Model 2 (q) with partial preservation of the structure of the fibrous ring (k, x), violation of the integrity of the locking plate (b, f, q, u). Hypertrophied chondrocytes (arrow) u). Crack between the plates in the outer part of the fibrous ring (arrow) (i, o, w, y). Accumulation of collagen fibers in the nucleus pulposis (arrow) next to clusters of chondrocytes (arrows) (d, g, t, v). Designation: fibrous ring (FR), nucleus pulposis (NP). Hematoxylin and eosin.

Table

Quantitative assessment of degenerative changes in intervertebral discs (in points) (according to A. Lai et al. [15])

Category	Model 1			Model 2		
	cranial	damaged	caudal	cranial	damaged	caudal
Nucleus pulposus						
Form	1.0 ± 0.6	—	1.4 ± 0.5	1.0 ± 0.0	—	0.7 ± 0.6
Area	0.7 ± 0.5	—	1.6 ± 0.5	0.3 ± 0.6	—	0.7 ± 0.6
Number of cells	1.0 ± 0.8	—	1.2 ± 0.8	1.0 ± 1.0	—	1.0 ± 1.0
Cell morphology	1.1 ± 0.7	—	1.2 ± 0.8	1.0 ± 1.0	—	1.0 ± 1.0
NP sum	3.9 ± 2.2	—	5.4 ± 2.1 p1 = 1.000	3.3 ± 2.5 p3 = 0.833	—	3.3 ± 2.5 p1 = 1.000 p3 = 0.250
NP – AF margin	1.6 ± 0.5	—	1.6 ± 0.9	0.7 ± 0.6	—	1.0 ± 1.0
Fibrous ring						
Lamellar structure	0.3 ± 0.5	2.0 ± 0.0	0.5 ± 0.5	0.0 ± 0.0	1.5 ± 1.0	0.0 ± 0.0
Cracks/ruptures	0.7 ± 0.5	1.7 ± 0.5	0.5 ± 0.5	0.3 ± 0.6	1.8 ± 0.5	0.3 ± 0.6
FR sum	1.0 ± 0.8	3.7 ± 0.5 p1 = 0.016 p2 = 0.026	1.0 ± 1.1 p1 = 0.564	0.3 ± 0.6 p3 = 0.267	3.3 ± 1.5 p1 = 0.157 p2 = 0.157 p3 = 1.000	0.3 ± 0.6 p1 = 1.000 p3 = 0.548
Endplate	0.3 ± 0.5	2.0 ± 0.0	0.5 ± 0.5	1.0 ± 0.0	2.0 ± 0.0	0.3 ± 0.6
Sum	6.7 ± 1.6	5.7 ± 0.5	7.3 ± 2.9 p1 = 1.000	5.3 ± 3.5 p3 = 0.517	5.3 ± 1.5 p3 = 1.000	5.0 ± 3.0 p1 = 0.655 p3 = 0.381

Notes: p1 — comparison with the corresponding indicator of the L_{IV}–L_V disc; p2 — comparison with the corresponding indicator of the L_{VI}–S_I disc; p3 — comparison with the corresponding indicators of Model 1. NP — nucleus pulposus, FR — fibrous ring.

bodies, as well as foci of newly formed spongy bone tissue (Fig. 5, b). The spinal nerves at this level were surrounded by an epineurium consisting of dense connective tissue with fibroblasts between the fibers (Fig. 3, c). In the nerves, similar degenerative changes in axons were detected, as in the case of ligation with a thread: amorphous decay products were found between the fibers, fragmentation of fibers, Schwann cells were rare (Fig. 3, c, d).

Caudal segment. In the fibrous ring, collagen fibers in 80 % of cases retained normal structural organization, only in one rat (20 %) signs of disorganization were detected. The cellular composition also corresponded to the norm and consisted of fibrochondrocytes and chondrocytes. The cell density was mainly average. In 40% of animals, interfibrous gaps were recorded between the plates of the fibrous ring, and in the others, the plates were intact (Fig. 2, y).

In the nucleus pulposis, the matrix was heterogeneously stained in 40 % of the individuals, with notochordal cells and chondrocytes in them, and with chondrocytes in the remaining rats with heterogeneously stained matrix. Signs of degeneration in the form of accumulation of collagen fibers were found in 40 % of cases (Fig. 2, v).

The endplates were intact in 80 % of the animals, with calcified only in one (20 %). In all rats, vascular sprouting was not recorded.

Signs of inflammation were also not detected in any case.

According to the assessment of degenerative changes in rats of Model 2, no significant difference was recorded between the cranial and caudal intervertebral discs, both in general assessment and separately for the annulus fibrosus and the nucleus pulposus (Table). The obtained values were equal to ≈ 5 points. When comparing the values of the annulus fibrosus of the damaged disc with the corresponding values for the cranial and caudal discs, no difference was also found. No difference was found between the models for all discs in the case of a quantitative assessment in the points.

Discussion

As a result of the study, we developed two models of degenerative diseases of the spine with compression of the spinal nerves and destruction of the nucleus pulposus: 1) without fixation of this spinal segment and with ligation of the spinal nerve in the LS with a thread; 2) with fixation of this spinal

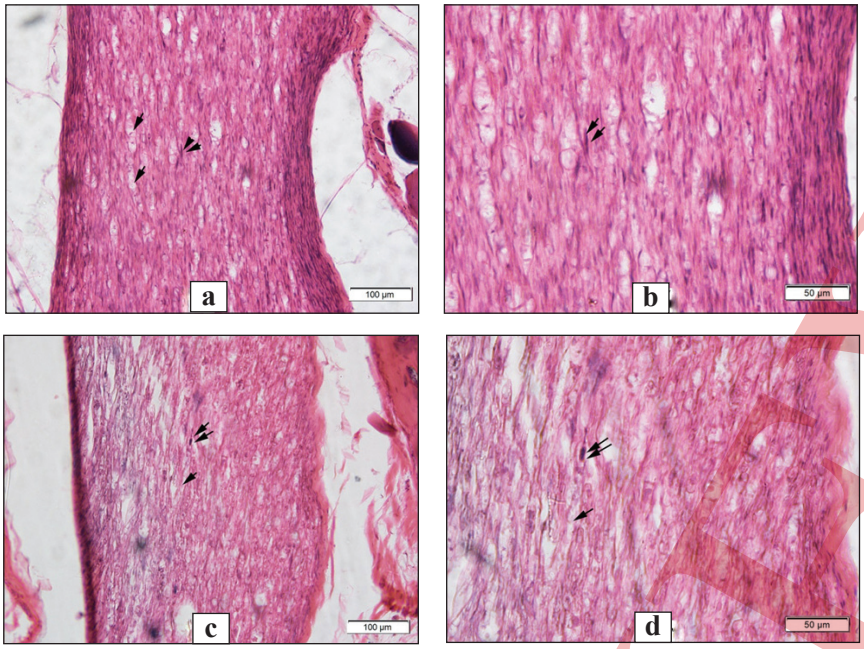

Fig. 3. Spinal nerve distal to nerve root clamp or nerve trunk ligation in rats in Models 1 (a, b) and 2 (c, d). Degenerative changes in axons (arrow) and single Schwann cells (arrows). Hematoxylin and eosin

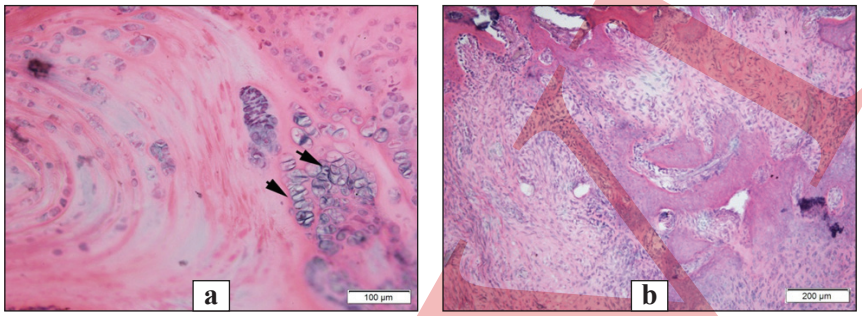

Fig. 4. Structural features of tissue formation in the area of destroyed nucleus pulposus in rats in Model 2. Replacement of the nucleus pulposus with cartilage tissue with hypertrophied chondrocytes (a) or connective tissue with bone formation (b). Hematoxylin and eosin

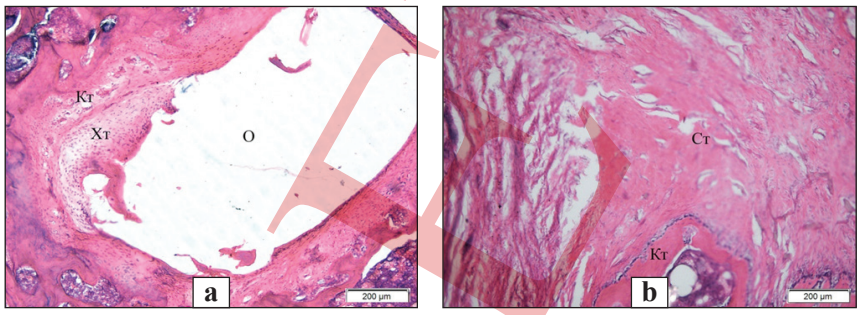

Fig. 5. Fragment of the vertebral body of a rat spinal segment with a destroyed nucleus pulposus in Model 2. Hole (H) from the end of the fixing bracket around which connective tissue (Ct), cartilage tissue (Cart) and bone tissue (Bt) have formed (a). Connective tissue is formed from the outside of the vertebral body and newly formed spongy bone tissue (b). Hematoxylin and eosin

segment with two clamps that pressed on the roots of the corresponding spinal nerves. Reconstruction of intervertebral disc degeneration in animals is often performed by destroying the nucleus pulposis, in particular by puncturing it [8, 16]. In Model 1, we reproduced intervertebral disc degeneration and neuropathy in patients with degenerative spinal diseases before performing decompression-stabilization interventions to analyze how this affects the adjacent segments. Their condition is known to be one of the factors that affect the success of subsequent decompression-stabilization interventions and spinal fusion [17, 18]. In Model 2, we attempted to reproduce the complications caused by spinal nerve root compression due to misplacement of transpedicular screws during decompression-stabilization procedures at the L_{IV}–L_V level or more distally, resulting in neuropathic pain in patients [1, 2, 19]. During nerve compression/ligation, we monitored the animals' limb responses to confirm the accuracy of the procedure. In the models of intervertebral disc degeneration with nerve compression that we developed and reproduced in rats, we found the development of degenerative changes in the discs of adjacent segments in

both quantitative and qualitative assessments. However, we did not find any differences in the degeneration score [15] between the models for all the intervertebral discs studied. However, structural changes differed between the models in the nucleus pulposus of the intervertebral disc of the spinal segment caudal to the level of injury. Thus, Model 1 showed more pronounced replacement of notochordal cells by chondrocytes, cell death, accumulation of collagen fibers in the matrix, and formation of cracks than Model 2. In a similar study in mice, instability was caused by resection of the posterior elements of the spinal segment of the LS, resulting in degeneration of the nucleus pulposus at the level of the injury, but the adjacent segments were not evaluated [20]. J. Sun et al. in rabbits that underwent spinal fusion recorded degenerative changes in the nucleus pulposus in the adjacent segments [21]. The changes we found may also be due to the fact that disc puncture in rats causes an increase in interleukins in the adjacent discs in the first 14 days after injury, which indicates inflammation [11].

Degenerative changes in adjacent intervertebral discs may also be due to impaired muscle innervation resulting from ligation of the nerve plexus or compression of the spinal nerve roots. Another factor may be dysfunction in the spinal nerve roots, which occurs due to increased interleukin levels in the surrounding tissues due to inflammation in the damaged disc [22]. This negatively affects peripheral nerve function in the LS of the rats. Thus, neuropathy may cause skeletal muscle myopathy in animals [23]. We have previously shown that the development of degenerative changes in the intervertebral discs of rats is associated with atrophic changes in the muscles in the LS paravertebral muscle ischemia model [10]. Thus, a difference was found between the models at the level of the damaged intervertebral disc. In animals from Model 1, where the spinal segment with a damaged intervertebral disc was not stabilized with staples, degenerative changes in the annulus fibrosus of the injured disc, as assessed by the score [15], were greater than in both adjacent discs, which was not found in Model 2. This may indicate the effect of instability on the damaged disc. A. J Michalek et al. [7] showed a 20 % decrease in disc stiffness under different loading conditions (compression and torsion) in a rat model of a punctured intervertebral disc, which may indicate a decrease in its ability to withstand loads. Loss of strength after puncture of the nucleus pulposus has also been found in a cadaveric intervertebral disc during axial loading [24]. Also, in rats of Model 1, in which the replacement of the nucleus pulposus with cartilage tissue was detected, large cracks were detected, which was not the case in Model 2. In our opinion, this may also be due to the presence of instability in Model 1 compared to Model 2. S. Liu et al. [25] also recorded the formation of cracks in the nucleus pulposus in a model of instability of the lumbar spinal segment in mice.

Peripheral nerve injury by compression with a ligature or ligation with a thread is quite common when studying nerve degeneration/regeneration in animal models [26]. A feature of spinal nerve roots is a thinner epinervium and a lower content of collagen fibers compared to peripheral nerves, so the above actions cause more damage [27]. It is known that peripheral nerve compression leads to edema, which causes axonal damage due to increased endoneurial fluid pressure [26, 27]. When peripheral nerves are ligated in rats, the distal parts of the nerve undergo Wallerian degeneration [27], which we observed in both models. We did not detect signs of inflammation or increased macrophage numbers after 8 weeks of modeling, which is likely due to a significant decrease in these manifestations 2 weeks after mechanical nerve injury in rodents [28].

Conclusions

Spinal nerve compression and damage to the nucleus pulposus of the lumbar intervertebral disc in rats leads to degenerative changes in the adjacent discs — cranial and caudal.

The developed rat models can be used to study methods for treating disc degeneration in patients with neurological complications after decompression-stabilizing spinal surgery.

Conflict of interest. The authors declare the absence of a conflict of interest.

Prospects for further research. Search for new methods for treating neurological complications after decompression-stabilizing spinal surgery using the developed models.

Information on funding. The study was conducted within the framework of the research work of the National Academy of Medical Sciences of Ukraine “To improve methods of surgical treatment of patients with degenerative-dystrophic diseases of the lumbar spine using the latest technologies” (state registration No. 0121U111749).

Authors' contribution. Barkov O. O. — concept and design, data analysis, writing a draft of the article, approval of the final version of the article; Nikolchenko O. O. — experimental modeling in rats, approval of the final version of the article; Samoilova K. M. — experimental modeling in rats, approval of the final version of the article; Maltseva V. E. — histological analysis, data analysis, writing a draft of the article, approval of the final version of the article; Danyshchuk Z. M. — histological analysis, approval of the final version of the article.

References

1. Tarawneh, A. M., & Salem, K. M. (2020). A systematic review and meta-analysis of randomized controlled trials comparing

the accuracy and clinical outcome of pedicle screw placement using robot-assisted technology and conventional freehand technique. *Global spine journal, 11*(4), 575–586. https://doi.org/10.1177/2192568220927713
2. Cho, J. H., Lee, J. H., Song, K., & Hong, J. (2017). Neuropathic pain after spinal surgery. *Asian spine journal, 11*(4), 642–652. https://doi.org/10.4184/asj.2017.11.4.642
3. Gao, S., Li, Z., Li, X., Rudd, S., Wang, H., Gao, Z., Ding, W., & Yang, S. (2023). The treatment effect of posterior lumbar fusion surgery on patients suffering from lumbar disc herniation concurrent with peroneal nerve paralysis. *Frontiers in surgery,* 9. https://doi.org/10.3389/fsurg.2022.1063528
4. Cristea, A., Heijnen, B. F., Park, S. W., Krutko, A., Santos, C., Senker, W., Arzoglou, V., & Pereira, P. (2025). Neuropathic pain appears to be the main symptom associated with higher disease burden and lower pain alleviation in degenerative lumbar disease fusion patients. *Brain and spine*, 5, 104224. https://doi.org/10.1016/j.bas.2025.104224
5. Cannizzaro, D., Anania, C. D., Safa, A., Zaed, I., Morenghi, M., Riva, M., Tomei, M., Pessina, F., Servadei, F., Ortolina, A., & Fornari, M. (2023). Lumbar adjacent segment degeneration after spinal fusion surgery: A systematic review and meta-analysis. *Journal of neurosurgical sciences, 67*(6). https://doi.org/10.23736/s0390-5616.22.05891-x
6. Kang, Y., Liu, C., Wang, M., Wang, C., Yan, Y., & Wang, W. (2021). A novel rat model of interbody fusion based on anterior lumbar corpectomy and fusion (ALCF). *BMC musculoskeletal disorders, 22*(1). https://doi.org/10.1186/s12891-021-04822-4
7. Michalek, A. J., Funabashi, K. L., & Iatridis, J. C. (2010). Needle puncture injury of the rat intervertebral disc affects torsional and compressive biomechanics differently. *European spine journal, 19*(12), 2110–2116. https://doi.org/10.1007/s00586-010-1473-z
8. Wawrose, R. A., Couch, B. K., Dombrowski, M., Chen, S. R., Oyekan, A., Dong, Q., Wang, D., Zhou, C., Chen, J., Modali, K., Johnson, M., Sedor-Schiffhauer, Z., Hitchens, T. K., Jin, T., Bell, K. M., Lee, J. Y., Sowa, G. A., & Vo, N. V. (2022). Percutaneous lumbar annular puncture: A rat model to study intervertebral disc degeneration and pain-related behavior. *JOR Spine, 5*(2), e1202. https://doi.org/10.1002/jsp2.1202.
9. Glaeser, J. D., Tawackoli, W., Ju, D. G., Yang, J. H., Kanim, L. E. A., Salehi, K., Yu, V., Saidara, E., Vit, J. P., Khnkoyan, Z., NaPier, Z., Stone, L. S., Bae, H. W., & Sheyn, D. (2020). Optimization of a rat lumbar IVD degeneration model for low back pain. *JOR Spine, 3*(2), e1092. https://doi.org/10.1002/jsp2.1092
10. Skidanov, A., Ashukina, N., Maltseva, V., Skidanov, M., Danyshchuk, Z., & Radchenko, V. (2024). The relationship between structural changes in paraspinal muscles and intervertebral disc and facet joint degeneration in the lumbar spine of rats. *Journal of orthopaedic surgery and research, 19*(1). https://doi.org/10.1186/s13018-024-04548-8
11. Li, Y., Dai, C., Wu, B., Yang, L., Yan, X., Liu, T., Chen, J., Zheng, Z., & Peng, B. (2024). Intervertebral disc injury triggers neurogenic inflammation of adjacent healthy discs. *Spine journal, 24*(8), 1527–1537. https://doi.org/10.1016/j.spinee.2024.04.002
12. Shehab, S., Hamad, M. I., & Emerald, B. S. (2025). A novel approach to completely alleviate peripheral neuropathic pain in human patients: Insights from preclinical data. *Frontiers in neuroanatomy,* 18. https://doi.org/10.3389/fnana.2024.1523095.
13. European convention for the protection of vertebrate animals used for research and other scientific purposes. (1986). https://zakon.rada.gov.ua/laws/show/994_137#Text
14. On the protection of animals from cruelty (2006). https://zakon.rada.gov.ua/laws/show/3447-15#Text
15. Lai, A., Gansau, J., Gullbrand, S. E., Crowley, J., Cunha, C., Dudli, S., Engiles, J. B., Fusellier, M., Goncalves, R. M., Nakashima, D., Okewunmi, J., Pelletier, M., Presciutti, S. M., Schol, J., Takeoka, Y., Yang, S., Yurube, T., Zhang, Y., & Iatridis, J. C. (2021). Development of a standardized histopathology scoring system for intervertebral disc degeneration in rat models: An initiative of the ORS spine section. *JOR Spine, 4*(2), e1150. https://doi.org/10.1002/jsp2.1150
16. Tian, T., Wang, H., Li, Z., Yang, S., & Ding, W. (2021). Intervertebral Disc Degeneration Induced by Needle Puncture and Ovariectomy: A Rat Coccygeal Model. *Biomed research international, 2021.* https://doi.org/10.1155/2021/5510124
17. Kim, J. Y., Ryu, D. S., Paik, H. K., Ahn, S. S., Kang, M. S., Kim, K. H., Park, J. Y., Chin, D. K., Kim, K. S., Cho, Y. E., & Kuh, S. U. (2016). Paraspinal muscle, facet joint, and disc problems: risk factors for adjacent segment degeneration after lumbar fusion. *Spine journal, 16*(7), 867–875. https://doi.org/10.1016/j.spinee.2016.03.010
18. Lau, K. K., Samartzis, D., To, N. S., Harada, G. K., An, H. S., & Wong, A. Y. (2021). Demographic, surgical, and radiographic risk factors for symptomatic adjacent segment disease after lumbar fusion. *Journal of Bone and joint surgery, 103*(15), 1438–1450. https://doi.org/10.2106/jbjs.20.00408
19. O'Brien, J. R. (2017). Nerve Injury in Lateral Lumbar Interbody Fusion. *Spine, 42*, S24. https://doi.org/10.1097/BRS.0000000000002034
20. Oichi, T., Taniguchi, Y., Soma, K., Chang, S. H., Yano, F., Tanaka, S., & Saito, T. (2018). A Mouse Intervertebral Disc Degeneration Model by Surgically Induced Instability. *Spine, 43*(10), E557–E564. https://doi.org/10.1097/BRS.0000000000002427
21. Sun, J., Chen, F., Wei, X., & Ou, Y. (2024). Establishment of a Rabbit Model of Adjacent Intervertebral Disk Degeneration After Lumbar Fusion and Fixation and Evaluation of Autophagy Factor Expression in Nucleus Pulposus Cells. *Orthopedics, 47*(4), e167–e173. https://doi.org/10.3928/01477447-20240424-04
22. Özaktay, C. A., Cavanaugh, J. M., Asik, I., DeLeo, J. A., & Weinstein, J. N. (2002). Dorsal root sensitivity to interleukin-1 beta, interleukin-6 and tumor necrosis factor in rats. *European spine journal, 11*(5), 467–475. https://doi.org/10.1007/s00586-002-0430-x
23. Jensen, V. F. H., Molck, A.-M., Soeborg, H., Nowak, J., Chapman, M., Lykkesfeldt, J., & Bogh, I. B. (2018). Proximal neuropathy and associated skeletal muscle changes resembling denervation atrophy in hindlimbs of chronic hypoglycaemic rats. *Basic & clinical pharmacology & toxicology, 122*(1), 165–175. https://doi.org/10.1111/bcpt.12870
24. Chow, N., Sinopoli, S. I., Whittal, M. C., Bednar, D. A., & Gregory, D. E. (2025). An investigation of the mechanism of adjacent segment disease in a porcine spine model. *Clinical biomechanics, 122*. https://doi.org/10.1016/j.clinbiomech.2025.106441
25. Liu, S., Sun, Y., Dong, J., & Bian, Q. (2021). A mouse model of lumbar spine instability. *Journal of visualized experiments, 2021*(170). https://doi.org/10.3791/61722
26. Dubový, P. (2011). Wallerian degeneration and peripheral nerve conditions for both axonal regeneration and neuropathic pain induction. *Annals of anatomy, 193*(4), 267–275. https://doi.org/10.1016/j.aanat.2011.02.011
27. Klusáková, I., & Dubový, P. (2009). Experimental models of peripheral neuropathic pain based on traumatic nerve injuries - An anatomical perspective. *Annals of anatomy, 191*(3), 248–259. https://doi.org/10.1016/j.aanat.2009.02.007
28. Mueller, M., Leonhard, C., Wacker, K., Ringelstein, E. B., Okabe, M., Hickey, W. F., & Kiefer, R. (2003). Macrophage response to peripheral nerve injury: The quantitative contribution of resident and hematogenous macrophages. *Laboratory investigation, 83*(2), 175–185. https://doi.org/10.1097/01.LAB.0000056993.28149.BF

The article has been sent to the editors	Received after review	Accepted for printing
03.07.2025	04.08.2025	07.08.2025

HISTOLOGICAL CHANGES IN ADJACENT INTERVERTEBRAL DISCS IN A RAT MODEL OF NUCLEUS PULPOSUS INJURY AND SPINAL NERVE COMPRESSION

O. O. Barkov, V. Ye. Maltseva, O. A. Nikolchenko, Z. M. Danyshchuk, K. M. Samoylova

Sytenko Institute of Spine and Joint Pathology National Academy of Medical Sciences of Ukraine, Kharkiv

✉ Oleksandr Barkov, MD, PhD in Traumatology and Orthopaedics: a.barkov.79@gmail.com; https://orcid.org/0000-0003-2161-416X

✉ Valentyna Maltseva, Phd in Biol. Sci.: maltseva.val.evg@gmail.com; https://orcid.org/0000-0002-9184-0536

✉ Olga Nikolchenko, PhD in Biol. Sci.: o_nicolchenko@ukr.net; https://orcid.org/0000-0001-9808-9485

✉ Zinayda Danyshchuk, MD: zinada1962@gmail.com; https://orcid.org/0000-0003-2968-3821

✉ Kateryna Samoylova: samoylova_e@ukr.net; https://orcid.org/0000-0003-1806-3467