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MODEL OF LUMBAR SPINAL STENOSIS IN THE EXPERIMENT

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Abstracts

The description of an experimental model of lumbar spinal stenosis on 20 rats. The experiment was symmetrical dissection of arc plates to the inside thin cortical layer plates, and then dissection of the latter. The middle part of the arc with the spinous processes of the vertebrae is separated from the rest of the arc, and articular processes. The separated middle part of the arc with yellow ligament is shifted in the ventral direction, reducing the size of the cavity of the spinal canal and fix the contacting bone edges with bone cement. Degenerative changes of the nerve roots were evaluated histologically by endoneural and epineural changes using a 7-point scale of G. Byrond and others.

In the studied group of animals 7 days after spinal canal stenosis simulations appeared degenerative changes of nerve fibers, but the degree is low, and there is virtually no endoneural inflammation. The epineurium determined expressed or gross changes, indicating epineural inflammatory processes. After 1 month. There appeared dystrophic and degenerative changes of nerve fibers of the overwhelming majority (over 75%). At a later date (3 months), endoneural change remained practically the same as in the 1th month after surgery, epineural violations were preserved, there were groups and single fibroblasts as a sign of epineural fibrosis, as well as portions of connective tissue neoplasms and hyalinosis.

Keywords: lumbar spinal stenosis, an experimental model.

Introduction

Lumbar spinal stenosis (LSS) is a major cause of pathological changes in the elements of the cauda equina, causing severe neurological syndromes: radiculopathy, radiculo-myeloischemia and neurogenic claudication. To study the pathophysiology and pathomorphology elements of cauda equina, experimental simulation of spinal canal (SC) stenosis is used. Adequate experimental model is also needed for testing the effects of drugs and treatments for SC stenosis.

Structural changes in the SC and its contents (cauda equina and spinal roots, vessels) are studied mainly in acute experiments with compression of spinal roots for just a few hours [3]. For large animals (dog, pig) a dosed compression of cauda equina is carried out with the help of cartridges for 7 days to 3 months [2].

K. Yashihara et al. [6,7] modeled SC stenosis of cats by inserting epidural silicone film. The authors performed laminectomy L4 and L5 of spine and injected square fragment of silicon film having a thickness of 0.3 mm and a side of a square of 3.5 mm into the rear epidural space. In recent years, M. Sekiguchi et al. [4], and then K.Watanabe et al. [5] modified the method of obtaining of modified SC stenosis in rats. The method includes the opening of rear access to interarticular parts of the lower edges of two adjacent vertebrae L4 and L5, and mechanical compression of the contents of a SC by forcing local reduction of its cross section. Then they remove the yellow ligament between the vertebrae L4 and L5 and inject into SC, into the epidural space under the arch vertebra L5, across the gap between the vertebrae silicone block with length of 4.0 mm, width of 1 mm and thickness of 0.9 mm so that it would consume about half of the anteroposterior diameter of the SC.

This method has significant disadvantages. Firstly, the surgical manipulations within the SC cavity, connected with the removal of the ligamentum flavum and the injection of an alien object into the SC - silicone block, injure its contents, including the epidural fat, epidural vessels and elements of the spinal cord which leads to undesirable side effects (bleeding, formation of adhesions, traumatic changes in the structure and function of the spinal cord). Second, injected into SC silicone block is not fixed and is free to move both along and across it, which frequently results in SC stenosis of uncontrolled localization and extent.

Although silicon is considered to be biologically inert material, but after 3 weeks postoperatively connective tissue capsule around a silicone block inputted into the epidural space is formed [5]. Subsequently, caused by silicone epidural fibrosis is significantly modified during SC stenosis.

The aforementioned disadvantages of this method significantly reduce the accuracy of its performance and recreation of controlled real conditions required for spinal canal stenosis.

The purpose of this project is to create a process of acquiring spinal stenosis canal in small laboratory animals, which would provide a topographically accurate recreation of the actual conditions of this pathology of the spine by minimizing the traumatic surgery, and it would exclude damage to the contents of the spinal canal and reduce the degree of risk of epidural fibrosis.

Material and Methods

The proposed method of experimental modeling of SC stenosis, designed on 20 adult Wistar rats. The survival rate was 95%. The radiological and histological studies performed in 1 week, 1 month and 3 months confirmed that formed under our model SC stenosis is strictly localized and its degree is adjustable. Adverse effects resulting from surgical procedures in the performance of a SC stenosis simulation with proposed method was not revealed.

Technology of proposed method of LSS experimental model in rats. Anesthesia by intraperitoneal injection of one of barbitural drugs (thiopental, pentobarbital) at a dose of 30-50 mg / kg. A longitudinal skin incision over the spinous processes (≈ 20 mm) L4 - L6 vertebrae. Skeletonizing arc of L5 vertebra. Plain cutters on the right and left of the spinous process of the longitudinally dissected L5 arc plate symmetrically at an angle of 15-35º with respect to the sagittal plane (Fig. 1).

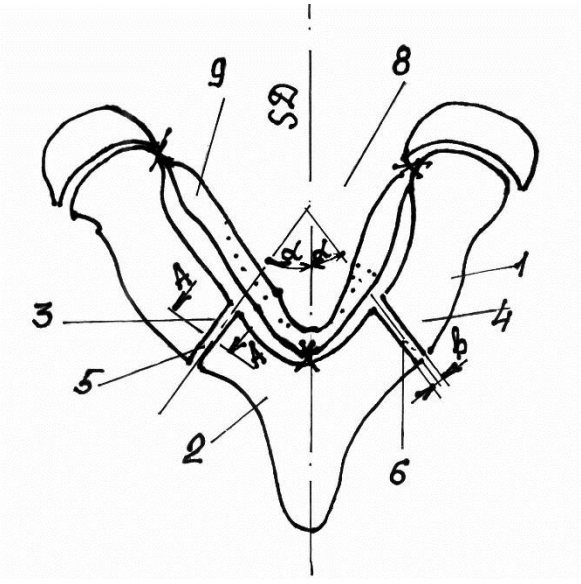
Symmetrical plates arc dissection is carried out in two stages: a cylindrical cutter makes grooves up to the internal thin plates of the cortical layer, and then using microsurgical scissors the latter are dissected. Thus, the middle portion of the arc with the spinous processes of the vertebrae are separated from the rest of the arc, and articular processes.

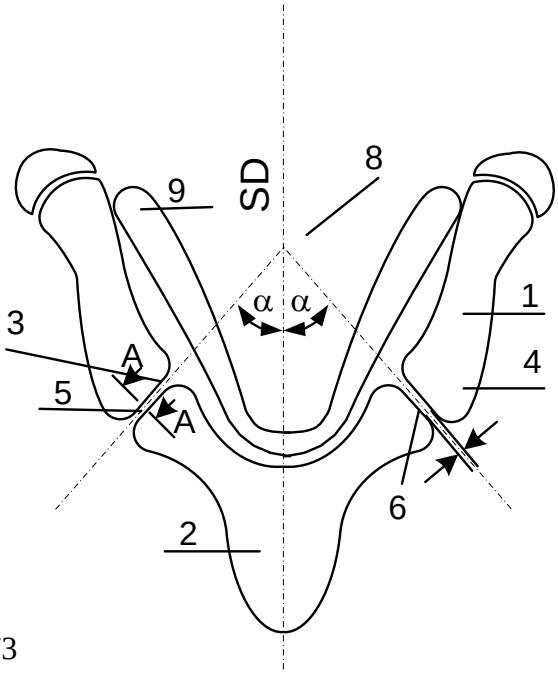
Separated middle portion of the arc with yellow ligament attached is shifted in the ventral direction, reducing the size of the SC cavity by the value determined by the stop of the side edges of the separate part of the arc to the side edges of the remaining part of the arc and fix the contacting edges of the bone with bone cement (Fig. 1b).

The magnitude of ventral displacement S of median separated part of the arc is calculated by the following formula:

$$S = b\sin\alpha,$$

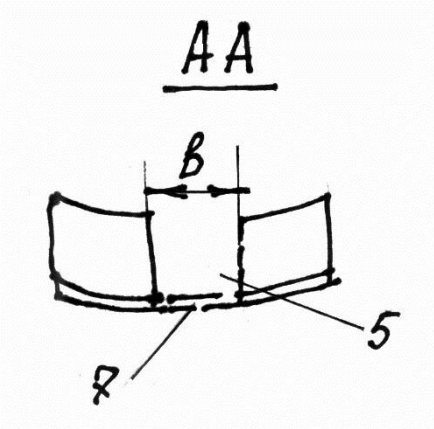
where S - the magnitude of ventral displacement of the middle separate part of the arc, which determines the degree of SC stenosis;





a

b



c

Fig. 1 Scheme method for simulation spinal canal stenosis:

a) general view of the arc in the axial projection: 1 - arc plate, 2 - separated middle part of the arc;
b) α - angle of inclination of the plane of each of the two sections of the arc plates relative to the sagittal plane;
c) b - the width of each of the two sections of the arc plates.

The offset S of the middle separated part of the arc vertebra results in reduction of the cross-section of SC. This decreases the anteroposterior dimension of the central portion, as well as its lateral sections (not shown in the figure) which makes it possible to model the central and lateral SC stenosis.

Yellow ligaments during dissection of the vertebral arch and during the ventral displacement of the separated middle part of the arc are not injured. Surgical manipulations in the cavity of SC are minimal and the injury of its contents is excluded. Selecting the angle of inclination α of the plane of each of the two sections of the plates of the arc to the sagittal plane in the range of from 15 to 35º and the width of each of the sections in the range of 1.0 to 1.5 mm, allowed to adjust the degree of SC stenosis in a quite wide range - from 15 up to 75%, which significantly expands the functionality of the modeling capabilities of SC stenosis.

For histopathological examination material was fixed with 10% neutral formalin, decalcified in EDTA salts during 7 - 10 days depending on the volume of the vertebra. Sections were deparaffinized with xylene, and stained with hematoxylin and eosin. Degenerative changes of the nerve roots were evaluated histologically for severity and endoneural epineural changes using a 7-point scale of Byrod et al. (1998) [1]:

0 st. - No damage to the nerve fibers;
1 st. - Damage to individual nerve fibers;
2 st. - Damage to about 10% of the nerve fibers;
3 st. – Damage to about 11-25% of the nerve fibers;
4 st. – Damage to 26-50% of the nerve fibers;
5 st. – Damage to 51-75% of the nerve fibers;
6 st. – Damage to 76-100% of the nerve fibers.

Other histological changes were assessed on a 5-point scale:

0 - no change;
1 - minor changes;
2 - light changes;
3 - significant changes;
4 - gross changes.

For studying and photographing an optical microscope Olympus CX-31, and the camera Olympus C5050Z were used.

Results

Fig. 2 shows the rat's L5 vertebra in axial projection prior to simulation (Fig. 2a) and after simulation of SC stenosis (Fig. 2b). As an illustration of the model adequacy, axial CT images of L5 vertebra were represented on one rat before and after surgery simulation (Fig. 3a, 3b).

Fig. 2a. Preparations of L5 vertebra of the rat prior to modeling SC stenosis

Fig. 2b. Preparations of L5 vertebra of the rat after modeling SC stenosis (reduction in cross-sectional of SC area by 50%)

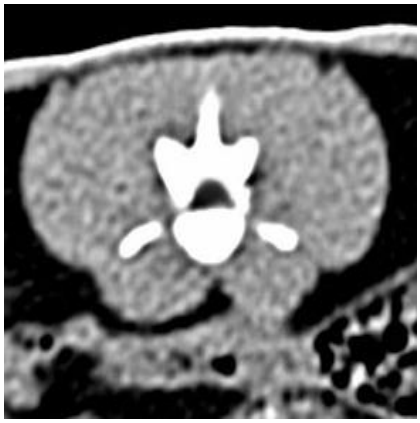


Fig. 3a. Axial CT scans of the rat prior to modeling SC stenosis.

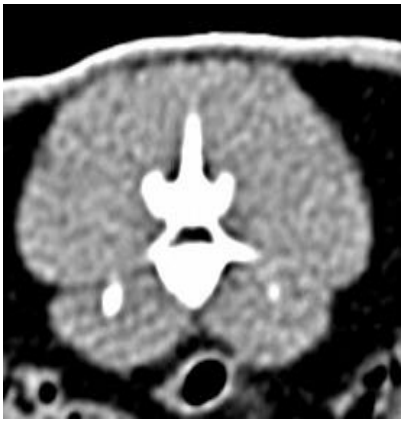


Fig. 3b. Axial CT scans of the rat after modeling SC stenosis.

Endoneural violations include: leukocyte infiltration, hemorrhage, congestion, edema of Schwann cells. Epidural changes: hyperemia, hemorrhage, fibroblasts, mast cells and leukocyte infiltration.

Epidural change was about widespread hemorrhages. Severe focal leukocyte infiltration, monocytes and mast cells were detected in isolated preparations. Epidural fibrosis was manifested by thickening and deformation of the meninges due to the formation of connective tissue with foci hyalinosis. Endoneural changes show signs of degeneration of the nerve fibers of the rear and front roots, swelling of the Schwann cells, signs of congestion and thrombosis of endoneural vessels (Fig. 4). There were also signs of endoneural inflammation (leukocyte infiltration, the presence of individual mast cells).

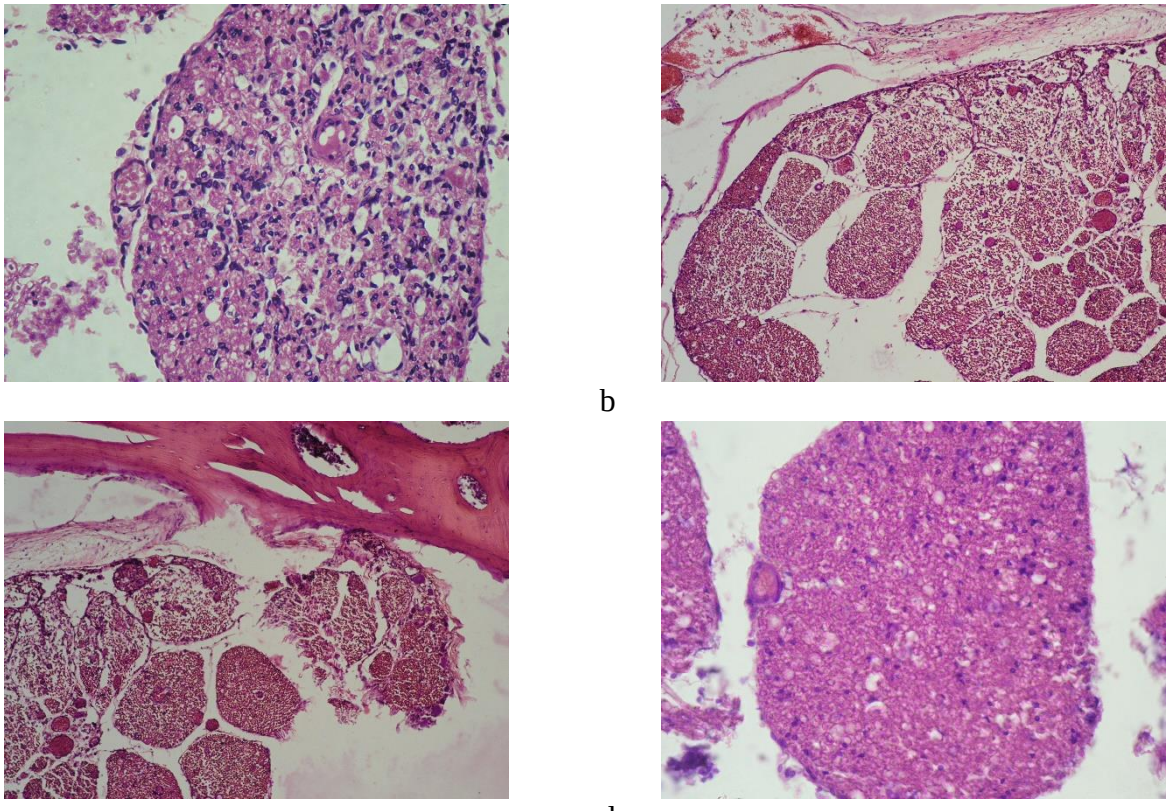


Fig. 4 endoneural changes: a) leukocyte infiltration; b) flushing; c) Schwann cell swelling; d) mast cells.

Table 1 shows average values of quantitative histologic, epineural and endoneural changes in spinal roots in the control group of animals and different terms after modeling SC stenosis.

Table1.

Mean values of quantitative endoneural and epineural changes in spinal roots (points) in rats in the control group and at different times after modeling SC stenosis.

Animal Group		endoneural changes					epidural changes				
		Damage to the Nerve fibers	leukocytic infiltration	hemorrhage	hyperemia	swelling of Schwann cells	hyperemia	hemorrhage	fibroblasts	mast cells	leukocytic infiltration
Control (n=2)		0	0	0	2,0	0	3,0	2,0	0	0	2,0
Experimental group	7th day (n=5)	2,6	0	1,4	2,4	1,2	4,0	3,6	0	2,4	3,2
	1 month. (n=5)	6,0	2,6	1,6	3,6	4,0	4,0	3,0	3,6	4,0	4,0
	3'd month. (n=5)	6,0	3,0	0	3,6	3,0	4,0	4,0	3,2	3,6	3,6

The table shows that in the control group of rats hyperemia of intraneural and epineural vessels was being determined, epineural hemorrhage and leukocyte infiltration of the fiber, which is likely to have arisen due to the surgical injury during laminectomy performed to extract dural tube with the elements of the cauda equina.

In the experimental group of animals, 7 days after modeling SC stenosis, degenerative changes of nerve fibers were detected, but their degree was small. Endoneural signs of inflammation were not observed (there was no leukocyte infiltration), but diapedetic hemorrhage and edema of Schwann cells were observed.

1 month after modeling SC stenosis, there were degenerative changes of the majority of nerve fibers (over 75%), leukocyte infiltration, stasis and hyperemia of endoneural vessels, spread hemorrhage, clear edema of Schwann cells. At a later time (3 months) endoneural changes were similar to those observed 1 month after surgery.

7 days after modeling SC stenosis, in epineurium were determined clear or gross changes: hyperemia, venous stasis, blood clots, common hemorrhages; mast cells and leukocyte infiltration, indicating an epineural inflammatory process.

In later periods (1 and 3 months.) described on the 7th day heavy epineural violations were observed. In addition, the new formation of connective tissue was accompanied by the formation of fibroblastic nodules and foci hyalinosis (5b).

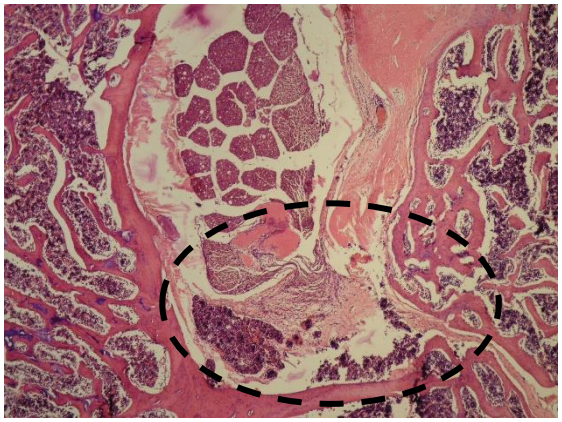


Fig. 5a. Coarse fiber connective tissue in the SC (circled line). H & E stain. Zoom - 100X.

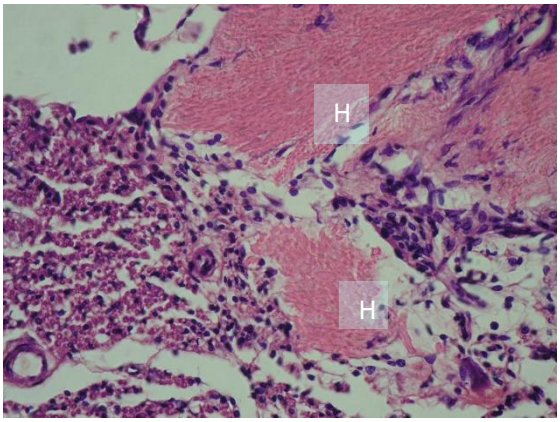


Fig. 5b. Outbreaks hyalinosis (D) to the PC. Hematoxylin and eosin. Zoom - 200X.

Conclusion

The experimental model of stenosis in spinal canal allows to adjust the degree of stenosis, significantly reduce the risk of uncontrolled damage to the contents of the SC and formation of epidural fibrosis. The method is easily reproduced, it can be used not only to study the pathogenesis of SC stenosis, but also to experimentally assess the effectiveness of drugs and treatments.

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