Experimental Model of Intervertebral Disk Mediated Postoperative Epidural Fibrosis

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Key Words

Experimental model · Postoperative epidural fibrosis ·
Laminectomy · Intervertebral disk

Abstract

Background: Postoperative epidural fibrosis (EF) after lumbar discectomy is the most common and at the same time controversial issue. **Purpose:** The etiology and pathogenesis creates a lot of discussion and selection of methods of treatment and prevention continues. **Methods:** L_{IV} laminectomy with dura mater (DM) exposition was done in 24 rats, and then, 0.3 ml of elements of suspension of autologous intervertebral disk was implicated on DM. As autologous intervertebral disk, we used the intervertebral disk from amputated tail. In all the animals, incisions were closed with 3/0 Vicryl. EF was examined. Fibroblast cell density was calculated in each field at ×40 magnification: Grade 1 – fewer than 100 fibroblasts in each field; Grade 2 – 100–150 fibroblasts in each field; Grade 3 – more than 150 fibroblasts in each field. **Results:** Based on histological results, we confirmed our model of experiment. On the 30th day of evaluation, there were significant histological evidences of postoperative epidural adhesions in experimental animals, which included the obliteration of epidural space, the presence of adhesions in

the dura and nerve roots, the restructuring of the yellow ligament, bone sclerosis, excessive appearance of fibrous tissue around the autologous intervertebral disk tissue that applied on the DM. **Conclusion:** In our work, we describe a new experimental model, where the elements of autologous intervertebral disk play the role of inflammation trigger, which cause postoperative scar and EF.

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Introduction

Surgical treatment of herniated lumbar disk is one of the most common spine operations. The procedure does not always result in pain relief, and epidural scar formation is one of the reasons why this treatment fails. Epidural fibrosis (EF) has been described in 24–38% of patients with failed back surgery syndrome [1–3]. Re-operations, aimed at adhesiolysis and scar resection are difficult and ineffective and have higher risk of complications [4–6]. Although surgical intervention is important for the pathogenesis of postsurgical forms of EF, some aspects of this disorder are difficult to explain based only on wound healing process [7, 8]. Literature data analysis shows that there are different inflammatory substances

Fig. 1. Block resected vertebral column with paraspinal muscle (between the Th_x and L_{V1} levels).

involved in formation of scar adhesions after spinal surgery, and various degrees of peridural fibrosis are detected [3, 9]. Therefore, the investigations and ongoing experimental studies of pathogenesis of postoperative epidural scar formation are issues of current interest of modern neurosurgery. They are necessary for further search for adequate model of intervertebral disk mediating postoperative EF and developing new methods of treatment and prevention of EF after intervertebral disk hernia surgery [10–12]. The purpose of our study was to work out a new experimental model of EF and identify factors triggering extensive scar formation after lumbar spine surgery. Model is necessary for the development of adequate methods of postoperative epidural scar prevention.

Methods

The study was approved by the Ethical Committee of Irkutsk Scientific Center of Surgery and Traumatology (No. 5, November 5, 2011). In this study, authors used 36 male Wistar rats, each weighing 250 ± 30 g, and the animals were allocated into 2 groups. Prior the operation, all rats endured intramuscular cephalosporin sodium injection (20 mg/kg). The animals were anesthetized with intraperitoneally administrated ketamine hydrochloride (2 mg/kg; Ketalar; Pfizer Inc., USA) and were fixed on the operation table in the prone position. Following sterile isolation, 3 cm midline surgical incisions were performed between the first and the fifth lumbar vertebrae. The paravertebral muscles were dissected, exposing L_{IV} laminae. For performing laminectomy, we used a high-speed electrical drill, and in all cases, dura mater (DM) was exposed. L_{IV} laminectomy with DM exposition was done in 24 rats, and then, 0.3 ml of elements of suspension of autologous intervertebral disk was implicated on DM. As autologous intervertebral disk, we used the intervertebral disk from amputated tail. The elements of autologous intervertebral disc were suspended and applied on DM. In all the animals, incisions were closed with 3/0

Vicryl. Then, the rats were left for free food and water consumption. Twelve rats were sacrificed 30 days after the surgery and 12 rats were sacrificed after 60 days. In the control group, L_{IV} laminectomy was performed in 12 rats. Six rats of the control group were sacrificed in 30 days after surgery and the rest were sacrificed in 60 days.

The animals were sacrificed with intraperitoneally administered thiopental sodium solution (10 mg/kg). After narcotization, all blood was removed from their abdominal aorta.

Then, we exposed the paravertebral region and resected the vertebral column including paraspinal muscle (between the Th_x and L_{V1} levels) in an en bloc fashion (fig. 1). The tissue samples were fixated with 10% formaldehyde solution and decalcified; 5- μ m-thick sections were stained with hematoxylin and eosin (H&E). The preparations were examined under a light microscope with magnification $\times 40$, 100, 200 and 400. EF was examined according to the following scheme designed by He et al. [10]: Grade 0 – DM is free of scar tissue; Grade 1 – only thin fibrous bands are observed between the scar tissue and DM; Grade 2 – continuous adherence is observed in less than two-thirds of the laminectomy defect; and Grade 3 – scar tissue adherence is large, affecting more than two-thirds of the laminectomy defect, or the adherence extended to the nerve roots (radix spinalis (RS) retraction). Fibroblast cell density was calculated in each field at $\times 40$ magnification: Grade 1 – <100 fibroblasts in each field; Grade 2 – $100\text{--}150$ fibroblasts in each field; Grade 3 – >150 fibroblasts in each field.

Statistical Analysis

We performed data analysis with the help of SPSS for Windows, version 11.5 (SPSS Inc., Chicago, Ill., USA). The Shapiro-Wilk test was used to determine normal distribution of continuous variables. The Bartlett test was used to evaluate the homogeneity of the variances. Continuous and ordinary variables were shown as medians (min–max). Non-parametric Kruskal–Wallis test determined the statistical significance of the EF extension (foreign-body reaction, granulation tissue, inflammation grading criteria: Grade 0 = DM is free of scar tissue; Grade 1 = minimal; Grade 2 = moderate; Grade 3 = extensive), inflammation, RS retraction, dural adhesion (dural adhesion grading scale: Grade 1 = only thin fibrous bands observed between DM and scar tissue; Grade 2 = continuous adherence observed in less than two-thirds

Table 1. Comparison of epidural fibrosis by grade number of rats (%)

Group	Grade 1	Grade 2	Grade 3
Experiment	1 (8.4)	4 (33.3)	7 (58.3)
Control	8 (66.7)	4 (33.3)	–

of the laminectomy site; Grade 3 = large scar tissue affecting more than two-thirds of the laminectomy site, or the adherence extends to the nerve roots), and fibroblast cell density among groups. To compare the differences in the median values among the groups, we used the Mann–Whitney U test. The likelihood ratio test was applied to determine whether the differences in nominal data were statistically significant. When the p values from the likelihood ratio test data were statistically significant, we employed Wilcoxon's exact test to determine which group differed from which of the other groups. A p value <0.05 was considered statistically significant.

Results

Histological assessment of the lumbar spine of rats under light microscopy revealed different changes in the surgical field (table 1).

Grade 1 of EF was marked in 66.7% of animals in the control group, which was characterized by the existence of only thin fibrous bands between scar tissue and dura-mater (fig. 2).

Moderate adhesions (Grade 2) were marked in 4 animals of the control group (fig. 3). It should be noted that none of the animals of the control group were observed having Grade 3 of EF.

It was established, that rough scar-adhesion changes prevailed in the experimental group. Pathological changes corresponding Grades 2 and 3 of EF were revealed in 11 animals. Moreover, in 58.3% of animals with elements of the autologous intervertebral disk as a trigger of EF, adhesions filled more than two-thirds of laminectomy space or spread to nerve roots (fig. 4). It was revealed that nerve roots adhered to DM in the spinal canal space and deformation of the dural sac with rough adhesive processes, and obliterated epidural space were marked. Also, the spinal canal defect was filled with lots of newly formed fibrous tissue, which was bounded on the sides by fragments of yellow ligament. All these facts pointed to rapid development and preservation of EF.

In the control group, Grade 1 dural adhesion was demonstrated in 9 rats (75%), and Grade 2 was found in the

Fig. 2. Grade 1 fibrosis as it is observed in the control group on the 30th day. Less EF and fibroblast cell density and only thin fibrous bands between DM and scar tissue are observed. H&E, original magnification $\times 40$.

Fig. 3. Grade 2 fibrosis as observed in the control group on the 30th day. EF adhere to the underlying DM. H&E, original magnification $\times 40$.

remaining 3 rats (25%). In the experimental group, Grade 1 dural adhesions were observed in 1 rat (8.3%); Grade 2 – in 3 rats (25.0%); and Grade 3 – in 8 rats (66.7%). In the control group, RS retraction was marked in 1 rat (12.5%). Whereas, in experimental group, dural adhesion with RS retraction were found in 7 rats (58.3%). Fibroblast cell density and EF were lower in the control group than in the experimental group ($p = 0.003$ and $p = 0.005$, respectively); these differences were statistically significant (fig. 5).

Fig. 4. Grade 3 fibrosis as observed in the experimental group on the 30th day. Dense EF, dural adhesion and RS and DM retraction are seen in the laminectomized area. H&E, original magnification $\times 40$.

Fig. 5. Graph of comparison of fibroblast cell density in the control and experimental groups.

There was significant concordance on all parameters between 2 observations (k coefficient 0.684, 0.712, 0.702 and 0.502 for EF, dural adhesion, fibroblast cell density and RS retraction, respectively). All these values were statistically significant ($p < 0.001$).

By the 60th day of the experiment, scar formations in animals of the experimental group progressed both in epidural space and in DM. Inflammation processes were

of multiform character, whereas in the control group, these changes were less expressed, which suggested reduction of inflammation.

Cellular reaction and fibroblast proliferation decreased in both groups and were less noticeable in the experimental group in 60 days. Hyalinized fibrous tissue, characterized by trabecular structure and osteoblastic activity, filled the laminectomy defect. Between the animals of the control group and the experimental group, we found important differences regarding the fibroblast cell density, and EF ($p = 0.002$ and $p = 0.034$, respectively). Moreover, inflammation, dural adhesion and fibroblast cell density were observed significantly less frequently in the control group than in the experimental group ($p = 0.04$, $p = 0.005$ and $p = 0.004$, respectively). These results showed statistically significant difference between the parameters of fibroblast cell density in the experimental and control groups ($p < 0.05$).

Discussion

It is known that scar tissue is always formed as a physiological reaction to any surgical intervention in response to the surgical trauma. However, the intensity and duration of this process may be different and depends on many factors. At present, the reasons of the EF cause a lot of discussion. LaRocca and Macnab [12] stated that post-operative hematoma in epidural space, which replaces epidural adipose tissue and eventually leads to development of aseptic inflammation, causes intensive scar formation and EF. Furthermore, migration of fibroblasts from affected paraspinal muscles results in enhanced collagen synthesis [6]. The ratio of cells and fibrous structures changes and friable connective tissue transforms into dense scar adhesions. According to some researchers, patrimonial factor associated with hyperergic reaction of fibroblasts as a response to surgical trauma plays an important role as well [13–15]. In addition, it is known that the tissue of degenerated nucleus pulposus can maintain a state of chronic inflammation in spinal canal and nerve roots, membranes of spinal cord and epidural adipose tissue, and it causes reactive changes therein which leads to development of scar adhesions [14, 16]. Intervertebral disk tissue is avascular; it is formed separately from the immune system and possesses antigenic properties. The destruction of intervertebral cartilage triggers the cascade mechanism of cellular immunity, which leads to formation of anti-disk antibodies. Antigen–antibody complexes stimulate the production of pro-inflammatory

substances (cytokines, prostaglandin E) and proteolytic enzymes (proteases, collagenases) that induces progressive degeneration of the intervertebral disk and adhesions development with other structures of the spinal canal [4, 7].

In modern science, researchers preserve high interest to this subject due to the fact that development of EF is one of the reasons of compression and fixation of neurovascular structures and circular stenosis of spinal canal, leading respectively to pain occurrence and neurological symptoms in patients undergoing spinal surgery. The fact that EF is one of the reasons of the failed back surgery syndrome is well known [6, 8]. Thus, according to various authors, postoperative epidural adhesions are responsible for up to 25% of reoperations [7, 8].

The mechanisms of development of postoperative epidural adhesions are still unclear. There is a question what the reasons are that some patients after spinal surgery have severe EF with appropriate symptoms while in other cases it is minimal despite the same conditions. Neurosurgical investigations of this issue are quite rare, so clinical and experimental experience is minor [16, 17], and further research in this area is required. Based on histological results, we confirmed our model of experiment. On the 30th day of evaluation, there were significant histological evidence of postoperative epidural adhesions in

experimental animals, which included the obliteration of epidural space, the presence of adhesions in the dura and nerve roots, the restructuring of the yellow ligament, bone sclerosis, excessive appearance of fibrous tissue around the autologous intervertebral disk tissue that applied on the DM.

In conclusion, we can resume that the proposed method of postoperative EF modeling can be widely used in experimental medicine for research of epidural scar adhesions pathogenesis and finding the ways of treatment and prevention of the disease in human.

Author Contributions

S.N.L. and V.A.S. designed the study, performed analysis and drafted the manuscript. K.C.E., S.A.L. and O.A.G. performed the histological and statistical analyses. S.N.L. and K.C.E. validated the pathological diagnosis. All the authors have read and approved the final manuscript.

Disclosure Statement

The authors have not disclosed any financial relationship with any company whose product might be affected by the research described or with any company that makes or markets a competing product.

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