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Intervertebral Disk Mediated Postoperative Epidural Fibrosis: Experimental Model and Methods of Prevention

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Abstract

The etiology and pathogenesis creates a lot of discussion, and selection of methods of treatment and prevention continues. In this study we used 36 male Wistar rats, each weighing 250 ± 30 g; LIV laminectomy with dura mater exposition was done in 12 rats, and then, 0.3 cc of elements of suspension of autologous intervertebral disk were implicated on dura mater. In 12 animals laminectomy was followed by application of a 5 × 6 mm sheet of Reperen™ on the exposed dura™. Histological assessment of the lumbar spine of rats observed that the elements of autologous intervertebral disk play the role of inflammation trigger, which cause postoperative scar and epidural fibrosis. In another part of the experimental study we revealed that Reperen™ to prevent excessive epidural fibrosis. So, the experimental model and methods of prevention postoperative epidural fibrosis can be widely used in experimental medicine for research of epidural scar-adhesion pathogenesis and for searching the ways of treatment and prevention of the disease in human.

Keywords: Experimental model; Postoperative epidural fibrosis; Laminectomy; Intervertebral disk

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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 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, have higher risk of complications and often are ineffective [4,5]. Although surgical intervention is important for the pathogenesis of postsurgical forms of epidural fibrosis, some aspects of this disorder are difficult to explain basing only on wound healing process [6,7]. Literature data analysis shows that there are different inflammatory substances involved in formation of scar adhesions after spinal surgery, and various degrees of peridural fibrosis are detected [2,8,9]. Therefore, the investigations and ongoing experimental studies of pathogenesis of postoperative epidural scar formation are issues of current interest in modern neurosurgery. They are necessary for further search for adequate model of intervertebral disk mediating postoperative epidural fibrosis and developing new methods of treatment and prevention of epidural fibrosis after intervertebral disk hernia surgery [10-13]. The purpose of our study was to work out a new experimental model of epidural fibrosis and its prevention. Model is necessary for the development of adequate methods of postoperative epidural scar prevention.

Materials and Methods

The study was approved by the Ethical Committee of Irkutsk Scientific Center of Surgery and Traumatology (N 5, 05.11.2011). In this study we used 36 male Wistar rats, each weighing 250±30 g, and the animals were allocated into three groups. Prior the operation all rats endured intramuscular cefazolin 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 LIV laminae. For performing laminectomy we used a high-speed electrical drill, and in all cases dura mater was exposed. LIV laminectomy with dura mater exposition was done in 12 rats (group 1), and then, 0.3 cc of elements of suspension of autologous intervertebral

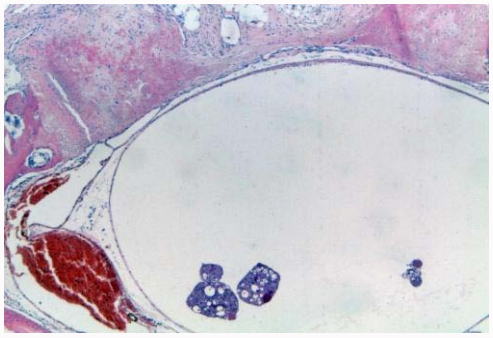

Figure 1: Grade 1 fibrosis as observed in the control group on the 30th day. Less EF and fibroblast cell density and only thin fibrous bands between DM and scar tissue are observed. Hematoxylin and Eosin, original magnification × 40.

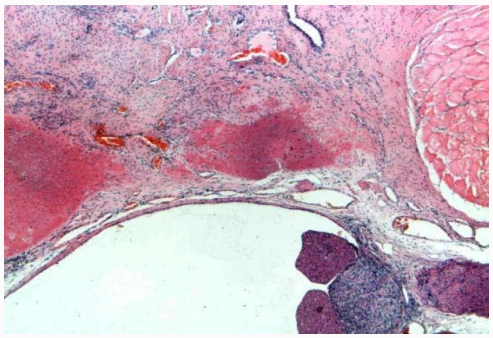

Figure 2: Grade 3 fibrosis as observed in the experimental group on the 30th day. Dense EF, dural adhesion, and RS and DM retraction are seen in the laminectomized area. Hematoxylin and Eosin, original magnification × 40.

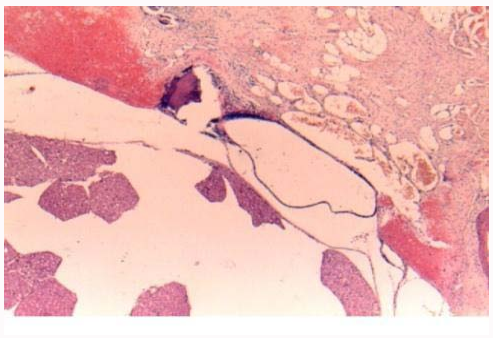

Figure 3: Grade 2 fibrosis as observed in the Reperen™-treated group on the 30th day. Hematoxylin and Eosin, original magnification × 40.

disk were implicated on dura mater (10). In group 2 of animals (n=12) laminectomy was followed by application of a 5 × 6 mm sheet of Reperen™ on the exposed dura, and then, 0.3 cc of elements of suspension of autologous intervertebral disk were implicated on the sheet of Reperen™. As an autologous intervertebral disk we used intervertebral disk from amputated tail. The elements of autologous intervertebral disc were suspended and applied on dura mater. In all animals incisions were closed with 3/0 vicryl. Then the rats were left for free food and water consumption. 6 rats in groups 1 and 2 were sacrificed 30 days after the surgery and the rest – after 60 days. In the control group, LIV laminectomy was performed in 12 rats. Six rats of the control group were sacrificed in 30 days after surgery and the rest – 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 paravertebral region and resected vertebral column including paraspinal muscle (between the ThX and LVI levels) in an en bloc fashion (8). The tissue samples were fixated with 10% formaldehyde solution and decalcified, 5 micron thick sections were stained with Hematoxylin-Eosin (H&E). The preparations were examined under a light microscope with magnification × 40, 100, 200 and 400. Epidural fibrosis was examined according to the following scheme designed by Y He et al. [10]: Grade 0 – dura mater is free of scar tissue; Grade 1 – only thin fibrous bands are observed between the scar tissue and dura mater; Grade 2 – continuous adherence is observed in < two-thirds of the laminectomy defect; and Grade 3 – scar tissue adherence is large, affecting > two-thirds of the laminectomy defect, or the adherence extended to the nerve roots (radix spinalis retraction) 10. 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.

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

Group	Grade 1	Grade 2	Grade 3
Group 1	1 (8.4%)	4 (33.3%)	7 (58.3%)
Group 2	7 (58.3%)	3 (25.0)	2 (16.7%)
Control	8 (66.7)	4 (33.3%)	–

Statistical analysis

We performed data analysis with the help of SPSS for Windows, version 11.5 [SPSS Inc., Chicago, IL, 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 ordinal variables were shown as medians (min-max). Nonparametric Kruskal-Wallis test determined the statistical significance of the epidural fibrosis extension*, inflammation, radix spinalis retraction, dural adhesion†, 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 less than 0.05 was considered statistically significant.

Results

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

Grade 1 of epidural fibrosis, marked only in one rat in group 1, and was characterized by the presence of only thin fibrous bands between scar tissue and dura mater (Figure 1).

It was established, that rough scar-adhesion changes prevailed in group 1. Pathological changes corresponding to Grade 2 and 3 of epidural fibrosis were revealed in 11 animals. Moreover, in 7 animals with elements of the autologous intervertebral disk as an epidural fibrosis trigger, adhesions filled more than 2/3 of laminectomy space or spread to nerve roots (Figure 2). It was revealed that nerve roots were adhered to dura mater 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, along the sides bounded by fragments of yellow ligament. All these facts pointed to rapid development and preservation of epidural fibrosis.

In the Reperen™-treated group of animals epidural scars were less extensive than in the group 1. Pathological changes corresponding to Grade 2 and 3 of epidural fibrosis were revealed in 5 animals. Grade 1 of epidural fibrosis, marked in 58.3% of animals in group 2, was characterized by the existence of only thin fibrous bands between scar tissue and the sheet of Reperen™ (Figure 3).

In the control group, Grade 1 dural adhesion was present in 9 rats (75%), and Grade 2 was found in the rest 3 rats (25%). In group 1, 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 Reperen™-treated group of rats, Grade 1 dural adhesions were observed in 7 rats (58.7%), Grade 2 – in 3 rats (25.0%), and Grade 3 – in 2 rats (16.7%). In the control group, radix spinalis retraction was marked in 1 rat (12.5%). Whereas, in group 1 dural adhesion with radix spinalis retraction were found in 7 rats (58.3%), but in the Reperen™-treated group they were detected in 2 animals (16.7%). Fibroblast cell density and epidural fibrosis were lower in group 2 than in group 1 ($p=0.003$, and $p=0.005$, respectively); these differences were statistically significant.

There was significant concordance of all parameters between control and group 1 observations (k coefficient 0.684, 0.712, 0.702, and 0.502 for epidural fibrosis, dural adhesion, fibroblast cell density and radix spinalis retraction, respectively). All of these values were statistically significant ($p< 0.001$).

By the 60th day of the experiment scar formations in animals of group 1 progressed both in epidural space and in dura mater. Inflammation processes were of multiform character. Whereas in the control and group 2 these changes were less expressed, which suggested reduction of inflammation.

Cellular reaction and fibroblast proliferation decreased in all groups and were less noticeable in group 1 in 60 days. Hyalinized fibrous tissue, characterized by trabecular structure and osteoblastic activity, filled the laminectomy defect. Between the animals of the Reperen™-treated group and of group 1 we found important differences regarding the fibroblast cell density and epidural fibrosis ($p=0.003$ and $p=0.035$, respectively). Moreover, inflammation, dural adhesion, and fibroblast cell density were observed significantly less frequently in the Reperen™-treated group than in group 1 ($p=0.042$, $p=0.005$, 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 epidural fibrosis cause a lot of discussion. La Rocca et al. [12]. stated that post-operative hematoma in epidural space that replaces epidural adipose tissue and eventually leads to aseptic inflammation, causes intensive scar formation and epidural fibrosis. Furthermore, migration of fibroblasts from affected paraspinal muscles results in enhanced collagen synthesis [13]. 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 [14-16]. In addition, it is known that 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 [14,17]. 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 proinflammatory substances (cytokines, prostaglandin E) and proteolytic enzymes (proteases, collagenases), and that induces progressive degeneration of the intervertebral disk and adhesions with other structures of the spinal canal [4,6].

In modern science, researchers preserve high interest to this subject due to the fact that development of epidural fibrosis 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 epidural fibrosis is one of the reasons of the failed back surgery syndrome is well known [2,5]. Thus, according to various authors, postoperative epidural adhesions are responsible for up to 25% of reoperations [6,8].

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 epidural fibrosis with appropriate symptoms while in other cases the fibrosis is minimal despite the same conditions. Neurosurgical investigations of this issue are quite rare, so clinical and experimental experience is minor [14-18], and further research in this area is required. Basing on histological results we confirmed our model of experiment. On the 30th day of evaluation there were significant histological evidences of post-operative 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 dura mater.

In another part of the experimental study we used Reperen™ to prevent excessive epidural fibrosis. The present study noted statistically significant differences ($p< 0.05$) between the Reperen™-treated group and group of animals with experimental epidural fibrosis in relation to both components – fibroblast cell density and epidural fibrosis.

As a potential sheet for human use, Reperen™ has a favorable profile; it has demonstrated no significant toxicity (in the form of foreign body reaction) in experimental subjects. Similarly, inflammation did not develop after implantation of Gore-Tex, Adcon-L, or vicryl.

Conclusion

We can resume that the proposed method of postoperative epidural fibrosis modeling can be widely used in experimental medicine for research of epidural scar-adhesion pathogenesis and for searching the ways of treatment and prevention of the disease in human. Moreover, Reperen™ has a positive effect on dural and neural structures in the vertebral canal after lumbar disk spine surgery. So, this material may be used as one of the methods to prevent excessive scar hypertrophy after intervertebral disk surgery.

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