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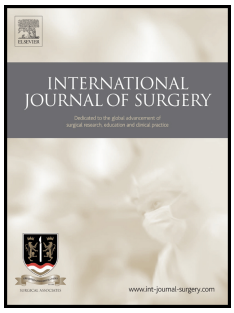

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Evaluation of Topical Dexmedetomidine Administration in Postlaminectomy Epidural Fibrosis Rat Model

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Evaluation of Topical Dexmedetomidine Administration in a Postlaminectomy Epidural Fibrosis Rat Model: Experimental Study

Abstract

Epidural fibrosis is a challenging topic in spinal surgery. Numerous clinical and experimental studies have been focused on this issue to clarify problems faced in spinal procedures for the patient as well as the surgeon and find out new methodologies. Dense cytokines and growth factors which are released from inflammatory cells have been suggested to play a major role in the inception and progression of fibrosis. One of the most investigated and important actor in epidural fibrosis is assumed to be the transforming growth factor-1ß (TGF-1ß) formation. Studies showed that Dexmedetomidine (DEX) downregulates TGF-ß pathway with its anti-inflammatory and antioxidant effects. From this point of view, for the first time in the literature we try to observe if there will be an effect of topical DEX administration over epidural fibrosis in a rat model. We hypothesized that DEX might have preventive effects on epidural fibrosis via anti-inflammatory and antioxidant effects. Twenty-four adult male Wistar albino rats were randomly assigned to three groups (Topical DEX, Spongostan, Laminectomy). A total laminectomy was performed at the L3-L5 level and then the ligamentum flavum and epidural fat tissue were cleared away from the surgical site. Histopathological assessment was performed postoperatively after 4 weeks. Our study revealed that topical DEX administration may have effects on reducing epidural fibrosis. Topical DEX administration may be helpful in preventing epidural fibrosis after laminectomy in rats through multiple anti-inflammatory and antioxidant mechanisms as well as through TGF -1ß pathway.

Keywords: epidural fibrosis, laminectomy, dexmedetomidine

Introduction

Epidural fibrosis is a challenging topic in spinal surgery. Numerous clinical and experimental studies have been focused on this issue to clarify problems faced in spinal procedures for the patient as well as the surgeon and find out new methodologies [1-14]. Besides the rapid improvement of technology and sophisticated surgical strategies in management of spinal disorders, epidural fibrosis still remains a conundrum. In clinical practice, epidural fibrosis is a part of "post-laminectomy syndrome" or "failed-back surgery", which leads to persistent back and leg pain in association with compression and/or stretching the nerve root or the Dura mater [1, 8, 15]. Approximately 8% to 48% of patients who underwent surgery for lumbar disc herniation experience post-laminectomy syndrome or failed-back surgery [16]. Revision surgeries after post-laminectomy syndrome have an increased risk of complications like epidural bleeding, Dural lacerations, nerve root injuries owing to epidural fibrosis [1,12].

The exact mechanism of action in epidural fibrosis is complex and remains uncertain. Excessive deposition of collagen, fibronectin, and dermatan sulfate known as "extracellular matrix", and decrease in the tissue cellularity result in epidural fibrosis [17, 18]. Miscellaneous cytokines and growth factors which are released from inflammatory cells have been suggested to play a major role in the inception and progression of fibrosis. One of the most investigated and important actor in epidural fibrosis is assumed to be the transforming growth factor-1ß (TGF-1ß) formation [18,19].

Dexmedetomidine (DEX) is an a2-adrenoceptor agonist with sedative, analgesic, sympatholytic properties [20, 21]. However, DEX is already in clinical use as a sedative for intensive care unit patients. Laboratory studies about neuroprotective, antioxidant and anti-inflammatory effects of DEX have also been reported [22-26]. Transforming growth factor is an important mediator in initiating epidural fibrosis formation, and it is shown that DEX

downregulates TGF-ß pathway [27]. In recent literature, the preventive effect of DEX on epidural fibrosis has never been researched in the context of a post-laminectomy rat model.

In this study, we try to examine whether topical DEX is effective in preventing epidural fibrosis.

Materials and Methods

Experimental groups, anesthesia and surgical procedure

Animal care and all of the experiments were adhered to the European Communities Council Directive of November 24, 1986 (86/609/EEC) related to the protection of animals for experimental use. All of the experimental procedures used in this investigation were reviewed and approved by the local ethical committee of the Ministry of Health. Twenty-four adult male Wistar albino rats weighing 250-350g were used. The rats were randomly assigned to three groups with 8 rats per group.

The groups were as following:

Group 1: Control (n=8); only a laminectomy was performed, as described below.

Group 2: Spongostan (n=8); a Spongostan (Ethicon; Ethicon Endo-Surgery, Inc., Cincinnati, OH, USA) was soaked with 2 cc/kg saline solution and was left on the Dura mater after laminectomy.

Group 3: DEX (n=8); 100µg/kg Dexmedetomidine (Precedex, Hospira, Lake Forest, IL, USA) was applied with a Spongostan soaked with 0.5 mL of saline solution and left on the Dura mater after laminectomy.

All of the rats were kept in environmentally controlled conditions at 22°C to 25°C, with appropriate humidity and a 12-hour light cycle. The rats were granted free access to food and water. The animals were anesthetized by an intraperitoneal injection of 10 mg/Kg Xylazine (Rompun, Bayer, Turkey) and 50 mg/Kg ketamine hydrochloride (Ketalar, Parke Davis, Turkey) and allowed to breathe spontaneously. The rats were placed in the prone position.

After their lower backs were shaved, the surgical sites were sterilized using Povidone. All of the surgical procedures were performed by the same surgeon (MEY). A longitudinal midline skin incision was performed at the L3–L5 level. The lumbosacral fascia was incised, the paravertebral muscles were dissected subperiosteally, and the L3–L5 laminae were exposed. A total laminectomy was performed at the L3-L5 level and then the ligamentum flavum and epidural fat tissue were cleared away from the surgical site. The Dura mater was fully exposed and left intact. Hemostasis was achieved using cotton pads. After the application of the topical agents, the wounds were closed in anatomical layers using the same 4-0 Prolene polypropylene sutures (Ethicon; Ethicon Endo-Surgery, Inc., Cincinnati, OH, USA). There were no complications, no wound infections, or any adverse effects observed relevant to DEX. All of these procedures were performed carefully using a surgical microscope (Zeiss OPMI 1; Carl Zeiss Meditec, Oberkochen, Germany) to avoid damage in neural tissues.

Histopathologic assessment

Histopathologic assessment was performed postoperatively after 4 weeks. Animals were killed by the administration of a lethal dose (200 mg/Kg) of pentobarbital (Nembutal; Oak Pharmaceuticals, Lake Forest, IL, USA). The bones of the lumbar area were removed ‘‘en bloc’’ in a manner that included the paraspinal muscles. The specimens were fixed in 10% buffered formalin for 1 week, and then were decalcified for 5 days in EDTA/Hydrochloric acid solution. The laminectomy site was identified and four 2-mm thick sections were obtained. Sections were embedded in paraffin and serial sections (5µm) were cut with microtome and stained with Hematoxylin Eosin (HE) and Masson’s Trichrome (MT). To evaluate fibrotic changes and for grading epidural fibrosis between the groups, Masson trichrome staining protocol was used [15]. All laminectomized spine sections were evaluated in a blinded manner by a pathologist under the Nikon Eclipse 80i light microscope as regards Dural thickness and epidural fibrosis. Quantitative morphometric analysis was performed on

sections using the Nikon Nis Elements D 3.1 Digital Analyzing System. Mean values were used for statistical evaluation. Epidural fibrosis was evaluated as described in the literature [28]. Grade 0: Dura mater is free of scar tissue, Grade 1: only thin fibrous bands are observed between the scar tissue and the Dura mater, 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. Thickening of the arachnoid and adherence to the Dura, defined as Arachnoid involvement was also evaluated in this study. Fibroblast and inflammatory cell density in the epidural space were measured in the two borders and center of the laminectomy field and the mean was calculated. The cell densities were graded as; Grade 1 for less than 100 fibroblast/inflammatory cells per 400 field, Grade 2 for 100-150 fibroblasts/inflammatory cells per 400 field and Grade 3 for more than 150 fibroblasts/inflammatory cells per 400 field [29,30].

Statistical analysis

Data analysis was performed using SPSS for Windows, version 11.5 (SPSS, Inc., Chicago, IL, USA). The Shapiro Wilk test was used to determine if the distributions of continuous variables were normal. The nonparametric Kruskal Wallis test was used to compare differences in groups, while the difference between subgroups was analyzed with the Mann Whitney U Test.

The presence of arachnoidal involvement was analyzed using a likelihood ratio test. A p value less than 0.05 was considered statistically significant.

Results

No mortality and morbidity related to the procedure occurred. All animals were ambulatory at the time of sacrifice. There was no wound infection, erythema or cerebrospinal fluid leakage observed during the study.

The mean thickness of Dura was 20.03 μm in DEX group, 22.34 μm in Spongostan group and 27.84 μm in Control group. The difference between the DEX and Control group and the difference between Spongostan and DEX group were statistically significant (p=0.005, p=0.005, respectively) (Table 1). There was no significantly difference between Spongostan and Control group (p=0,114) **(Fig.1-A)**. Comparative analysis between groups is summarized in Table 2 and 3.

In DEX group Grade 1 epidural fibrosis was observed in 7 rats (87.5%) and Grade 2 epidural fibrosis was observed in 1 rat (12.5%). There was no Grade 3 epidural fibrosis in the DEX group. In the control group Grade 2 epidural fibrosis was observed in 1 rat (12.5%) whereas Grade 3 epidural fibrosis was observed in 7 rats (87.5%). In the Spongostan group, 2 rats were observed in Grade 1 (25%) and in Grade 2 (25%). Four rats were observed in grade 3 (50%). Difference between DEX and Spongostan group as well as DEX and Control group was statistically significant (DEX-Spongostan p<0.001, DEX-Control p<0.001) (Table 2) **(Fig.1-B)**. No statistically significant difference was determined between the Spongostan and Control group (p=0.095). **(Fig.2)**

In the DEX group, Grade 1 fibroblastic density was found in all of the rats. In Spongostan group, Grade 1 and Grade 2 fibroblastic density was observed in 4 (50%), 4 (50%) rats, respectively. Grade 2 fibroblastic density was found in all of the rats in the Control group. The differences between the DEX and control group, DEX and Spongostan group, Spongostan and Control groups were statistically significant (p<0.001, p<0.001 and p=0.025, respectively). **(Fig.1-C)**

In the DEX group, Grade 1 inflammatory cell density was seen in all of the 8 rats. In the Spongostan group, Grade 1 and Grade 2 inflammatory cell density was observed in 2 (25%), 6 (75%) of the rats, respectively. Grade 2 inflammatory cell density in 4 rats (50%) and Grade 3 inflammatory cell density in 4 rats (50%) was found in the Control group. When the

mean grade of the DEX group was compared with Control and Spongostan groups, the differences were statistically significant ($p<0.001$, $p<0.001$, respectively) (Table 2). The difference between the Spongostan and Control group was statistically significant ($p=0.015$) (Table 2). **(Fig.1-D)**

Arachnoidal involvement was observed in one rat (12.5%) in the DEX group. Three rats (37.5%) in the Spongostan group and 5 rats (62.5%) in the Control group showed arachnoidal involvement (Table 3). The differences between all groups weren't statistically significant ($p=0.102$, Likelihood ratio test, Table 3).

Discussion

Despite the improvement in surgical techniques and sophisticated treatment modalities in spinal surgery, failed back surgery is still a challenge. One of the most important factor in failed back surgery or post-laminectomy syndrome is epidural fibrosis [1,2]. Epidural fibrosis formation is a reason for low back and leg pain as a result of tractions of the Dura and nerve roots. Proliferation of fibroblasts, transformation of fibroblasts to myoblasts and accumulation of extracellular matrix is the main pathophysiology of Epidural fibrosis [19]. TGF -1ß is produced by t-lymphocytes, macrophages, smooth muscle cells, endothelial cells, fibroblasts, epithelial cells, and fibrocytes and is one of the most important mediator involved in the mechanism of epidural fibrosis [17, 19, 27, 31]. The fibroblasts produce the most important amount of TGF -1ß in comparison the other cells [32]. The increase in the TGF -1ß amount stimulates fibroblast proliferation, differentiation to myofibroblasts and accelerates the deposition of extracellular matrix [19,33]. Abnormal deposition of fibronectin produced by myofibroblasts induces fibrosis [19, 34]. Although this cascade is thought to be the explanation of epidural fibrosis after laminectomy, many other cytokines or mediators especially tumor necrosis factor alpha stimulates the proliferation of fibroblasts via TGF -1ß [19, 35].

Besides the sedative, analgesic, sympatholytic effects, several studies demonstrate the protective effect of DEX on ischemic brain, liver, kidney and intestine injuries, as well as ischemia/reperfusion (I/R)-induced acute kidney injury related lung injuries [22-24,26, 36-38]. A kidney I/R injury study exerts that DEX preconditioning ameliorates the injury and inflammatory response through p38-CD44-pathway [40]. Down regulation of the extracellular matrix-receptor interaction pathway in which CD44 gene plays a critical role in cell adhesion and promotes the transendothelial migration of leukocytes to injured tissue. Besides the decrease in CD44 expression, several proinflammatory pathways like TNF alpha were also inhibited by DEX [38, 40]. TNF is a potent activator of cJun N-terminal kinase, p38MAPK and NF-KB named as “the intracellular signaling molecules”, which regulates the expression of type 1 collagen alpha 1, interstitial collagenase and cytokines. Increase in the release of endothelin-1, and expression of adhesion molecules (E-Selectin, ICAM-1, VCAM-1) from endothelial cells is another role of TNF alpha which stimulates the proliferation of fibroblasts via TGF-1 ß [31]. Herein, Kuru et al reported that DEX had significant preventive effects on intraabdominal adhesions in rats due to anti-inflammatory and antioxidant effects [25]. In line with the previous studies, we try to understand the effect of DEX over epidural fibrosis which has not been studied in the relevant literature. Due to the fact that DEX was effective in preventing intraabdominal adhesions in rats we hypothesized that DEX might have also preventive effects on epidural fibrosis via the before reported anti-inflammatory and antioxidant effects. We used topical DEX in a rat laminectomy model and compare these results with the Control and Spongostan groups. As we see, the mean thickness of the Dura in the DEX group was significantly lower than the Spongostan and the Control group. The histopathologic results showed that laminectomy alone or laminectomy with Spongostan administration leads to higher epidural fibrosis in comparison to laminectomy with DEX administration 4 weeks after surgery. Another histopathologic parameter, Fibroblast cell

density was less significant in the DEX group, compared to laminectomy and Spongostan group. As reported in literature, fibroblasts produce the most important amount of TGF -1ß compared to other inflammatory cells [35]. Reducing the fibroblast cell density in the surgical area will also decrease the TGF -1ß pathway and this mechanism will effect epidural fibrosis formation [41]. Our results are in correlation with the abovementioned mechanism and demonstrate that DEX administration prevents migration of fibroblasts to the surgical area and this may cause a looser scar tissue with very low adherence to the Dura. Besides, inflammatory cell density and arachnoidal involvement were lower in the DEX group compared with the laminectomy and Spongostan group. All of these results suggest topical DEX administration has favorable effects on epidural fibrosis in laminectomized rats.

However, this study has some limitations. The number of rats in each group should be increased to obtain much more accurate results. In addition to that, detailed biochemical analyses, Real time PCR studies to examine mRNA levels of TGF -1ß may provide more exact results in future studies. On the other hand, comparative studies with systemic DEX administration and/or other molecules that have proven in preventing epidural fibrosis [1] should be designed to get much more **exact** results.

Conclusion

Our study suggests that DEX may have preventive effects on epidural fibrosis in this rat model. On the contrary the histopathological results revealed that laminectomy caused significant epidural fibrosis. Fibroblast cell density might be a good marker in showing the severity of epidural fibrosis. Fibroblast cell density was less significant in the DEX group, and this was in coherence with other studies in literature [42]. Blinded microscopic evaluation of the specimens by a pathologist showed a lower epidural fibrosis grade in DEX group compared with the laminectomy as well as the Spongostan group.

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Conflict of Interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Animal Experiments

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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Figure Legends

Figure 1: (A) Comparison of Dural thickness among groups. The horizontal lines in the middle of each box indicate the median, whereas the top and bottom borders of the box mark the 25th and 75th percentiles, respectively. The whiskers above and below the box mark the maximum and minimum Dural thickness. (B) Comparison of epidural fibrosis grades among groups. The horizontal lines in the middle of each box indicate the median, whereas the top and bottom borders of the box mark the 25th and 75th percentiles, respectively. The whiskers above and below the box mark the maximum and minimum epidural fibrosis grades. Asterisks represent extreme cases. (C) Comparison of fibroblastic density grades among groups. The horizontal lines in the middle of each box indicate the median, whereas the top and bottom borders of the box mark the 25th and 75th percentiles, respectively. The whiskers above and below the box mark the maximum and minimum fibroblastic density grades. (D) Comparison of inflammatory cell density grades among groups. The horizontal lines in the middle of each box indicate the median, whereas the top and bottom borders of the box mark the 25th and 75th percentiles, respectively. The whiskers above and below the box mark the maximum and minimum inflammatory cell density grades.

Figure 2: Photomicrographs of the study (Masson's Trichrome, x40 objective) (A) Grade 3 epidural fibrosis (EF) from Control group. EF covered the whole laminectomy defect and adhered to the underlying Dura mater (black arrow). Grade 3 fibrosis is observed in most of the specimens in Control group. (B) Grade 2 epidural fibrosis specimen from Spongostan group. Epidural fibrosis adhered the underlying Dura (black arrow) and covered less than two-thirds of the laminectomy defect. (C) In the DEX group Grade 1 fibrosis is adherent to the underlying Dura (black arrow) without direct contact to the medulla spinalis (MS).

Table 1: Histopathologic evaluation results among the study groups

Variables	Control	Spongostan	Dexmedetomidine	P value[1]
Dural thickness (μm)	27.84(22.40-35.29)[b]	22.34(20.12-30.02)[a]	20.03(10.02-30.02)[a,b]	0.005
Epidural fibrosis grade	3(2-3)[b]	3(1-3)[a]	1(1-2)[a,b]	0.001
Fibroblastic density	2[b,c]	2(1-2)[a,c]	1[a,b]	<0.001
Inflammatory cell density	3(2-3)[b,c]	2(1-2)[a,c]	1[a,b]	<0.001
Arachnoidal involvement (+/-)	5/3	3/5	1/7	0.129

Data were represented as median (25-75 percentile).

[1] Kruskal-Wallis test

[a] Dexmedetomidine group versus the spongostan group (p<0,01)

[b] Dexmedetomidine group versus the control group (p<0,01)

[c] Spongostan group versus the control group (p<0,05)

Table 2: Comparison of the Control, Spongostan and Dex Group

P- Value [2]	Thickness of Dura	Epidural fibrosis Grade	Fibroblastic density Grade	Inflammatory cell density Grade
Control vs Spongostan	0.114	0.095	0.025	0.015
Control vs DEX	0.005	<0.001	<0.001	<0.001
Spongostan vs DEX	0.05	<0.001	0.025	<0.001

[2] **Mann Whitney U test**

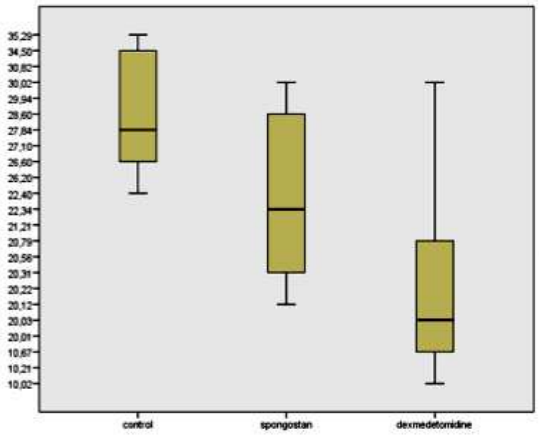
Table 3: Histological results of arachnoidal involvement

Groups	**Arachnoidal Involvement[a]**
Control	5/8 (62.5%)
Spongostan	3/8 (37.5%)
DEX	1/8 (12.5)
P value[b]	0.102

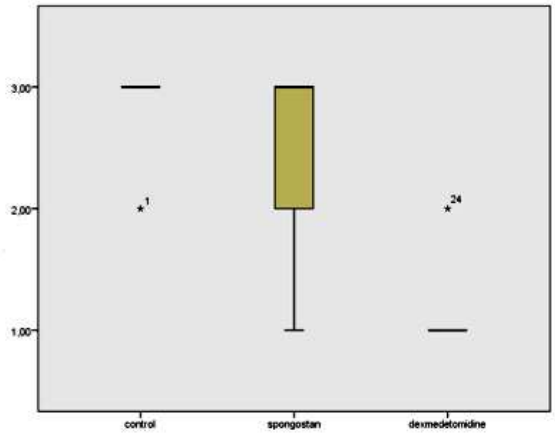
[a] **Data was represented number of rats and percentage (%)**

[b] **Likelihood Ratio Test**

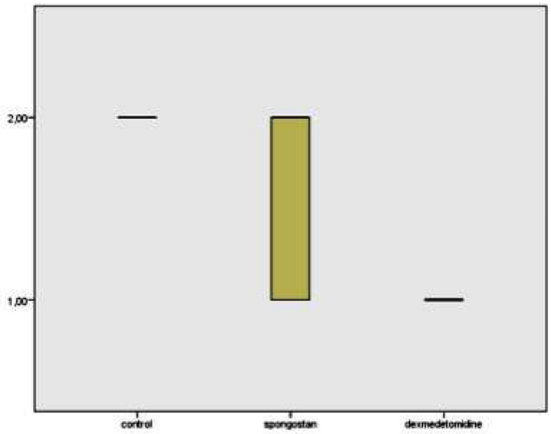
A. Dural Thickness (μm)



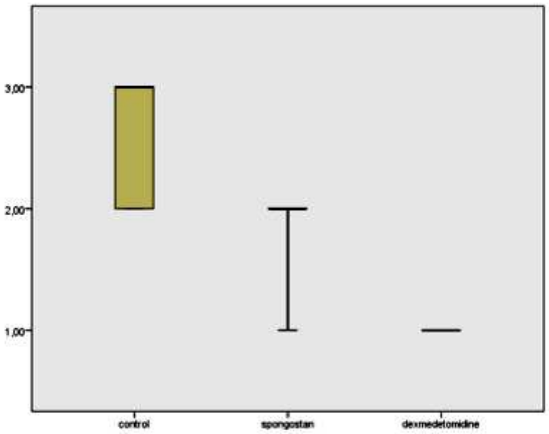
B. Epidural Fibrosis Grade

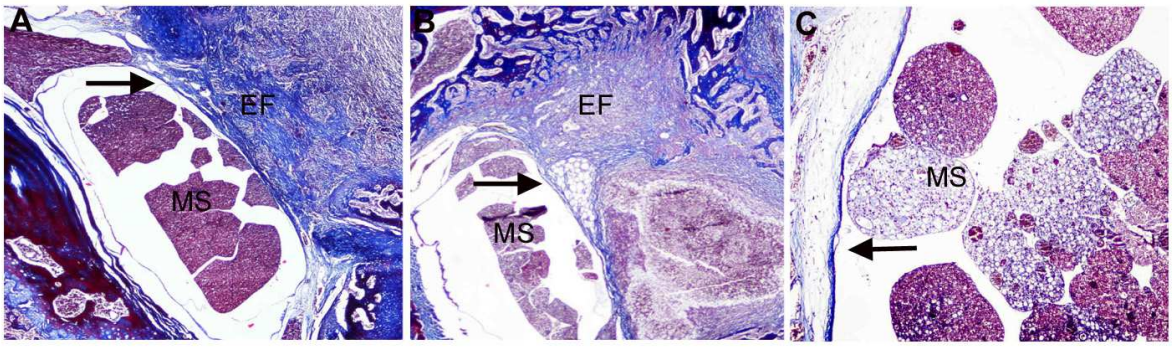


C. Fibroblastic density



D. Inflammatory cell density





Highlights

- Epidural fibrosis is a challenging topic in spinal surgery and the exact mechanism of action is still under investigation.
- Besides the clinical use as a sedative in intensive care units, laboratory studies about neuroprotective, antioxidant and anti-inflammatory effects of Dexmedetomidine have also been reported.
- The effect of Dexmedetomidine on epidural fibrosis has never been researched.
- DEX may have preventive effects on epidural fibrosis rat model.