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Basic Science

Evaluation of topical application and systemic administration of rosuvastatin in preventing epidural fibrosis in rats

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Abstract

BACKGROUND CONTEXT: Epidural fibrosis is a major challenge in spine surgery, with some patients having recurrent symptoms secondary to excessive formation of scar tissue resulting in neurologic compression. One of the most important factors initiating the epidural fibrosis is assumed to be the transforming growth factor-1β (TGF-1β). Rosuvastatin (ROS) has shown to demonstrate preventive effects over fibrosis via inhibiting the TGF-1β.

PURPOSE: We hypothesized that ROS might have preventive effects over epidural fibrosis through the inhibition of TGF-1β pathways.

STUDY DESIGN: Experimental animal study.

METHODS: Forty-eight adult male Wistar Albino rats were equally and randomly divided into four groups (laminectomy, spongostan, topical ROS, and systemic ROS). Laminectomy was performed at the L3 level in all rats. Four weeks later, the extent of epidural fibrosis was assessed both macroscopically and histopathologically.

RESULTS: Our data revealed that topical application and systemic administration of ROS both were effective in reducing epidural fibrosis formation. Furthermore, the systemic administration of ROS yielded better results than topical application.

CONCLUSIONS: Both topical application and systemic administration of ROS show meaningful preventive effects over epidural fibrosis through multiple mechanisms. The results of our study provide the first experimental evidence of the preventive effects of ROS over epidural fibrosis.

Keywords: Epidural fibrosis; Laminectomy; Rat; Rosuvastatin; Systemic; Topical

Introduction

Laminectomy is widely accepted choice of treatment in lumbosacral disorders, such as lumbar disc herniation. Unsatisfactory results may occur after laminectomy. Failed-back surgery syndrome is characterized by long-term unsatisfactory relief or recurrence of symptoms in patients who had laminectomies performed [1,2]. About 8% to 48% of patients who underwent lumbar disc surgery suffered from failed-back surgery syndrome [3–5].

Epidural fibrosis is a major challenge in spine surgery, with some patients having recurrent symptoms secondary to excessive formation of scar tissue resulting in neurologic compression [6,7]. The formation of epidural fibrosis causes compression and stretching of the associated nerve roots, leading to persistent back and leg pain [8,9]. Furthermore, postoperative epidural fibrosis may result in increased complications in revision surgeries, such as inadvertent dural lacerations, nerve root injuries, and epidural bleeding [10,11]. There is

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Conflict of interest: None.

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no way of predicting the patients who will develop symptomatic epidural fibrosis; once the condition occurs, there is no effective treatment [1].

The underlying mechanisms causing epidural fibrosis are complex. Epidural fibrosis results in a reduction of the tissue cellularity and excessive deposition of extracellular matrix (ECM) components such as collagen, fibronectin, and dermatan sulfate [12,13]. One of the most important factors initiating the epidural fibrosis is assumed to be the transforming growth factor-1β (TGF-1β) formation [11,14,15].

Statins, structural analogs of 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase, are currently used for the treatment of hyperlipidemia and the prevention of cardiovascular disease [16]. Aside from their antilipidemic effects, statins have been suggested to have effects on preventing fibrosis [17–27].

Rosuvastatin (ROS), a relatively new HMG-CoA reductase inhibitor, has exhibited a more potent affinity to HMG-CoA reductase and has the longest half-life compared with other statins [28]. Transforming growth factor-1β plays an important role in the formation of epidural fibrosis and ROS has shown to demonstrate preventive effects over fibrosis via the inhibition of TGF-1β [21,23,24]. In the current literature, preventive effects of ROS have never been studied in the postlaminectomy epidural fibrosis model.

In the present study, we use a rat laminectomy model to examine the effects of both topical application and long-term systemic administration of ROS on the prevention of epidural fibrosis.

Materials and methods

Experimental groups

Animal care and all of the experiments 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 ethical committee of the Ministry of Health Ankara Education and Research Hospital. Forty-eight adult male Wistar albino rats weighing 250±60 g were used. The rats were randomly assigned to four groups with 12 rats per group.

The groups were as follows:

Group 1: Laminectomy (n=12); only a laminectomy was performed, as described in the next section.

Group 2: Spongostan (n=12); 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: Topical ROS (n=12); 20 mg/Kg ROS (AstraZeneca, Cheshire, UK) was applied with a spongostan soaked with 0.5 mL of saline solution and left on the dura mater after laminectomy.

Group 4: Systemic ROS (n=12); laminectomy was performed as described in the next section and 20 mg/Kg ROS was administered daily through an intragastric tube for 4 weeks starting the day after laminectomy.

Anesthesia and spinal cord injury procedure

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 (Ketalar, Parke Davis, Turkey) and allowed to breathe spontaneously. A rectal probe was inserted and the animals were positioned on a heating pad to maintain their body temperature at 37°C.

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 (BG). A longitudinal midline skin incision was performed over the L2–L4 levels. The lumbosacral fascia was incised, the paravertebral muscles were dissected subperiosteally, and the L2–L4 laminae were exposed. A total laminectomy was performed at the L3 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 prolen polypropylene sutures (Ethicon; Ethicon Endo-Surgery, Inc., Cincinnati, OH, USA). There were no complications, no wound infections, or any adverse effects observed relevant to ROS. All of these procedures were performed carefully using a surgical microscope (Zeiss OPMI 1; Carl Zeiss Meditec, Oberkochen, Germany) so as not to injure the neural tissues.

Macroscopic assessment of epidural scar adhesion

Macroscopic assessment was performed after 4 weeks. Six rats were selected from each group and anesthetized by an intraperitoneal injection of 10 mg/Kg xylazine and 50 mg/Kg ketamine. The surgical sites were reopened carefully and epidural scar adhesion was evaluated by a professional neurosurgeon blinded to the treatment groups according to the Rydell classification [29]. This classification scheme includes the following grades: Grade 0: epidural scar tissue was not adherent to the dura mater, Grade 1: epidural scar tissue was adherent to the dura mater, but easily dissected, Grade 2: epidural scar tissue was adherent to the dura mater and dissected with difficulty without disrupting the dura mater, and Grade 3: epidural scar tissue was firmly adherent to the dura mater and could not be dissected.

Histopathologic assessment

Histopathologic assessment was performed postoperatively after 4 weeks. Six animals from each group 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 immersed into 10% buffered formalin. The spine was then further cut axially through the upper L2 to lower L4 levels to isolate the laminectomy. All of the specimens were sent for histologic evaluation. Histologic processes consisted of decalcification, dehydration, and preparation of paraffin-embedded blocks. Sections of 10 μm were cut on the axial plane and stained with Masson trichrome. Sections were examined using a Leica DM 600B microscope (Leica Microsystems, Mannheim, Germany) and photographed using a Leica DFC 490 camera (Leica, Wetzlar, Germany). All of the laminectomy sections were evaluated in a blinded manner by a professional histopathologist who analyzed the dural thickness and epidural fibrosis grades. Quantitative morphometric analysis was performed on sections using the Leica Application Suite Digital Analyzing System. Measurements were conducted at a magnification of ×40.

The thickness of the dura mater was measured at three points. The first sample was harvested from the midpoint of the laminectomy defect, the second sample was obtained 2 mm from the right side of the first sample, and the third sample was obtained 2 mm from the left side of the first sample. Mean values were used for statistical evaluation.

Next, epidural fibrosis was graded based on the scheme devised by He et al. [9]: 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.

In the normal tissues, arachnoid is thin and not adherent to the dura mater. When the arachnoid is thickened and adhered to the dura, this situation is defined as arachnoidal involvement, and the presence of the arachnoidal involvement was also noted.

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 Levene test was used to evaluate the homogeneity of the variances. Continuous and ordinal variables were shown as medians (minimum–maximum). The differences in the median values among the groups were compared using a Kruskal-Wallis test. When the p values from Kruskal-Wallis test statistics were statistically significant, we used Conover nonparametric multiple comparison test to determine which group differed from which other groups. We used the likelihood ratio test to determine whether the differences in nominal data were statistically significant. When the p values from the likelihood ratio test statistics were statistically significant, we used Fisher exact test to determine which group differed from which other groups. A p value less than .05 was considered statistically significant.

Results

Wound healing and complications related to the procedure

No mortality or morbidity occurred related to the procedure. Treatment with ROS had no adverse effects on the surrounding tissue or on wound healing in any rat. We did not observe wound infections, erythema, hematomas, or cerebrospinal fluid leaks. All of the animals were ambulatory at the time that they were killed.

Macroscopic assessment of epidural scar adhesion

After laminectomy, severe epidural adhesions (83.3% Grade 3 and 16.7% Grade 2) were observed in the laminectomy group. Likewise, in the spongostan group, all of the adhesions were Grade 3 (50%) or Grade 2 (50%). Comparing the mean grades of the laminectomy and the spongostan groups, there was a statistically significant difference ($p=.014$). Both the topical application and the systemic administration of ROS revealed soft or weak fibrous adhesions in the laminectomy sites. When the mean grades of the topical and systemic ROS groups were compared with the laminectomy group, the differences were statistically significant ($p<.001$ for both). Similarly, when the mean grades of the topical and systemic ROS groups were compared with the spongostan group, the differences were statistically significant ($p<.001$ for both). Therefore, both topical application and systemic administration of ROS prevented epidural fibrosis macroscopically. Furthermore, systemic administration of ROS showed statistically significant better results than topical application ($p=.003$) (Fig. 1, A).

Histopathologic assessment

The mean thicknesses of the dura mater were 15.2 μm in the laminectomy group and 14.5 μm in the spongostan group. In the topical ROS group, the mean thickness of the dura mater was 10.0 μm and in the systemic ROS group was 8.8 μm. The difference between the laminectomy and spongostan groups was not statistically significant ($p=.407$). The topical ROS group showed a statistically significant smaller dural thickness compared with both the laminectomy and spongostan groups ($p<.001$ for both).

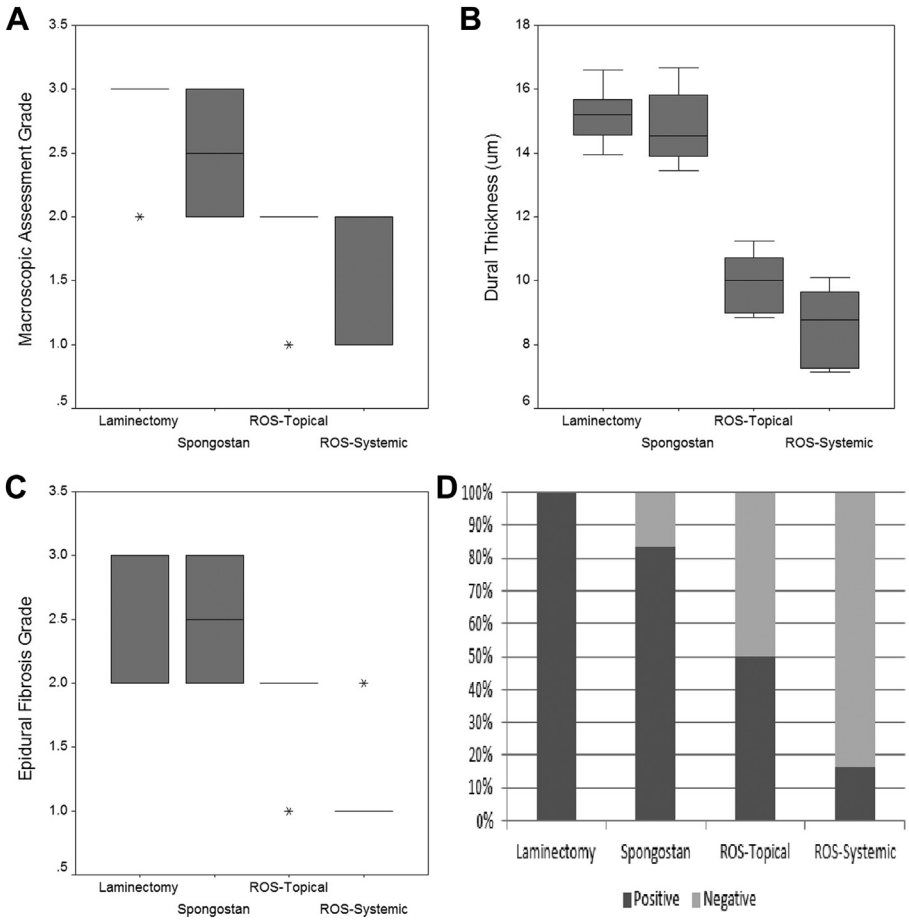

Fig. 1. (A) Comparison of macroscopic assessment grades among groups. The horizontal lines in the middle of each box indicate the median, whereas the top and bottom borders of the box mark the 25th and 75th percentiles, respectively. The whiskers above and below the box mark the maximum and minimum macroscopic assessment grades. Asterisks represent extreme cases. (B) 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. Asterisks represent extreme cases. (C) 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. (D) Comparison of arachnoidal involvement among groups. The light bars indicate the ratio of arachnoidal involvement negative within each group, the prevalence of arachnoidal involvement positive was shown as solid bars. ROS, rosuvastatin.

Similarly, the systemic ROS group showed a statistically significant smaller dural thickness compared with both the laminectomy and spongostan groups ($p<.001$ for both). Furthermore, the systemic ROS group showed a statistically significant smaller dural thickness compared with the topical ROS group ($p=.008$) (Fig. 1, B).

In the laminectomy group (Fig. 2, A), Grade 2 and 3 epidural fibrosis were observed in 33.3% and 66.7% of the rats, respectively. In the spongostan group (Fig. 2, B), Grade 2 and 3 epidural fibrosis were observed in 50% of the rats, respectively. There was no Grade 3 epidural fibrosis observed in either the topical or systemic ROS groups. In the topical ROS group (Fig. 2, C), Grade 1 and 2 epidural fibrosis were observed in 16.6% and 83.4% of the rats, respectively. In the systemic ROS group (Fig. 2, D), Grade 1 and 2 epidural fibrosis were observed in 83.4% and 16.6% of the rats, respectively. Epidural fibrosis grades of the topical ROS group were statistically significantly lower than the grades of both the laminectomy and the spongostan groups ($p<.001$ for both). As expected, the epidural fibrosis grades of the systemic ROS group were statistically significantly lower than the grades of both the laminectomy and the spongostan groups ($p<.001$ for both). Moreover, the epidural fibrosis grades of the systemic ROS group were statistically significantly lower than the grades of the topical ROS group ($p<.001$). No statistically significant difference was determined between the laminectomy and the spongostan groups ($p=.218$) (Fig. 1, C).

Arachnoidal involvement was observed in all of the rats in the laminectomy group and 83.4% of the rats in the spongostan group. Fewer rats in both the topical (50%) and the systemic ROS groups (16.6%) demonstrated arachnoidal involvement. The difference between the topical ROS and both the laminectomy and spongostan groups was not statistically significant ($p=.091$ and $p=.273$, respectively). Furthermore, the difference between the systemic ROS

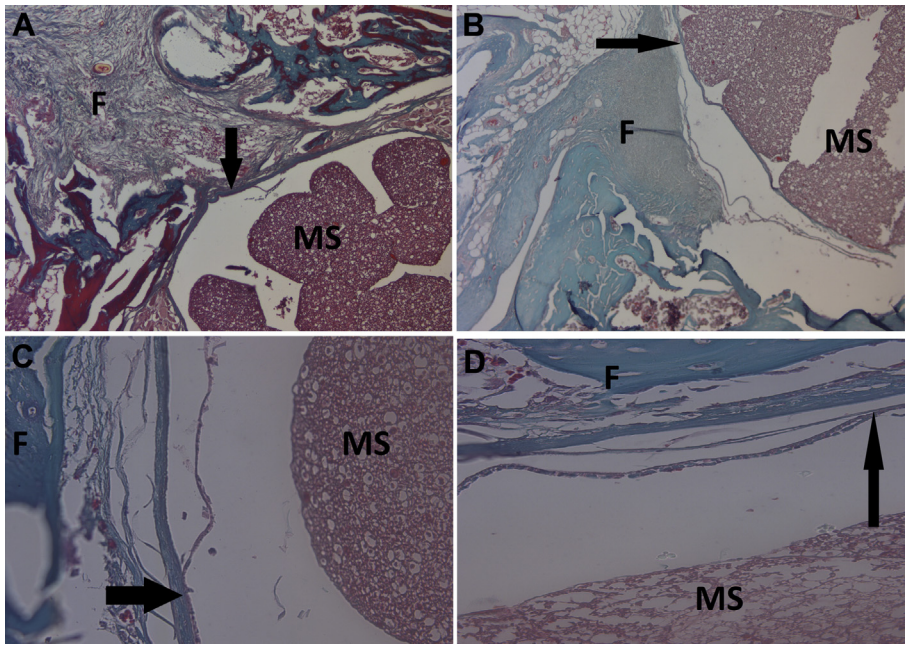

Fig. 2. Photomicrographs of the epidural fibrosis analysis of the study groups (Masson trichrome, ×40 objective). (A) In the control group, most of the specimens revealed Grade 3 fibrosis. The epidural fibrosis (F) completely covered the laminectomy defects and adhered to the underlying dura (black arrow). There is a direct contact observed between the fibrotic tissue and MS. (B) In the spongostan group, Grade 2 fibrosis is observed. The epidural fibrosis (F) adhered to the underlying dura (black arrow) and covered less than two-thirds of the laminectomy defects. Both in the (C) topical and (D) systemic rosuvastatin groups, better epidural fibrosis grades are observed. Only thin epidural fibrosis (F) is adherent to the underlying dura (black arrow). No direct contact was evident between the fibrotic tissue and the underlying MS. MS, medulla spinalis.

and both the laminectomy and spongostan groups was statistically significant (p=.008 and p=.04, respectively). On the other hand, there was no statistically significant difference between the systemic and the topical ROS groups for arachnoidal involvement (p=.273) (Fig. 1, D). The results of the study are summarized in the Table.

Discussion

Epidural fibrosis is one of the most common postoperative problems associated with spinal surgery. Epidural fibrosis was first discussed in 1948 [30]. However, since that time, there has been no effective treatment. The prevention of such scar formation can be achieved by meticulous hemostasis, minimal tissue trauma, and sterile surgical techniques.

The formation of epidural scar tissue is an expected postlaminectomy consequence, causing tractions on the dura mater and nerve roots that may result in lower back and leg pain [31,32]. Epidural fibrosis results from the proliferation of fibroblasts, transformation of fibroblasts to myoblasts, and the accumulation of the disorganized ECM proteins [11].

Previous experimental studies have demonstrated the beneficial fibrinolytic effects of statins in numerous fibrosis models [17–27]. Since statins have been shown to have preventive effects over fibrosis, we studied here the effects of topical application and systemic administration of ROS, a

Table
Macroscopic and histopathologic evaluation results among the study groups

Variables	Laminectomy	Spongostan	ROS-topical	ROS-systemic	p
Macroscopic assessment grade	3 (2–3)[*,†,‡]	2.5 (2–3)[*,§,¶]	2 (1–2)[†,§,‖]	1 (1–2)[‡,¶,‖]	.002
Dural thickness (μm)	15.2 (13.9–16.6)[†,‡]	14.5 (13.4–16.7)[§,¶]	10.0 (8.8–11.2)[†,§,‖]	8.8 (7.1–10.1)[‡,¶,‖]	<.001
Epidural fibrosis grade	3 (2–3)[†,‡]	2.5 (2–3)[§,¶]	2 (1–2)[† ,§,‖]	1 (1–2)[‡,¶,‖]	.002
Arachnoidal involvement (−/+)	0/6[‡]	1/5	3/3	5/1[‡]	.006

ROS, rosuvastatin.

Note: The variables are shown as median (minimum–maximum).

* Laminectomy versus spongostane (p=.014).

† Laminectomy versus ROS-topical (p<.001).

‡ Laminectomy versus ROS-systemic (p<.05).

§ Spongostane versus ROS-topical (p<.001).

¶ Spongostane versus ROS-systemic (p<.001).

‖ ROS-topical versus ROS-systemic (p<.01).

novel and potent statin [33]. Rosuvastatin had not been previously studied in the experimental epidural fibrosis model. Rosuvastatin does not require hepatic metabolism for activation and has a rapid bioavailability [33]. The dosage of the ROS used in this study was based on pharmacologic data from other rat studies; an oral dose of 12 mg/Kg with an $ED_{50\ (\text{median effective dose})}$ of 0.8 mg/Kg inhibits cholesterol synthesis completely [28]. Furthermore, other fibrosis studies relevant to ROS suggested the dose used in this study [20,34].

One of the most important mechanisms involved in epidural fibrosis is the formation of TGF-1β, which regulates essential cellular mechanisms [15]. All of the major cell types that are responsible for wound repair, such as T-lymphocytes, macrophages, smooth muscle cells, endothelial cells, fibroblasts, epithelial cells, and fibrocysts, produce TGF-1β [35,36]. Among these cells, fibroblasts are the predominant cell types involved in wound healing and produce increasing amounts of TGF-1β [37]. Excessive TGF-1β stimulates fibroblast proliferation, differentiation to myofibroblasts, and accelerates the deposition of ECM [38]. Additionally, myofibroblasts cause an abnormal deposition of ECM proteins such as fibronectin, resulting in fibrosis [39]. This cascade through TGF-1β is thought to have resulted in excessive epidural fibrosis in laminectomized spines [11].

It has been shown that ROS reduced TGF-1β formation and TGF-1β induced the expression of fibronectin [23,24]. In another study, Hermida et al. [21] reported that the fibrinolytic effects of ROS were based on the inhibition of TGF-1β formation. Therefore, we hypothesized that ROS might have preventive effects over epidural fibrosis via the inhibition of TGF-1β pathways.

Connective tissue growth factor is another widely considered universal mediator of the fibrinogenesis [40,41]. Connective tissue growth factor acts as a downstream mediator of cellular effect of TGF-1β in many cell types and stimulates fibronectin synthesis [42]. Rosuvastatin has been reported to inhibit TGF-1β-induced connective tissue growth factor and fibronectin expression [23]. This mechanism provides additional evidence for the fibrinolytic activities of ROS. Moreover, several other studies of fibrosis have shown that ROS exhibited preventive effects over fibrosis via multiple mechanisms, rather than TGF-1β-induced pathways. These effects included activation of peroxidase proliferation activated receptor-α, the augmentation of prostaglandin I2 and the reduction of prostaglandin E2, activation of AMP-activated (adenosine monophosphate) protein kinase, an increase in tissue-type plasminogen activator, and the inhibition of plasminogen activator inhibitor [17,21,23,43,44].

Since ROS was shown to have preventive effects over fibrosis in numerous studies, we used both topical application and systemic administration of ROS in an epidural fibrosis model in rats. Macroscopic assessment of the study groups revealed that both topical application and systemic administration of ROS protected the spine from epidural fibrosis. Daily systemic administration of ROS revealed better macroscopic results than the single topical application.

Masson trichrome is a three-color staining protocol that is often used to evaluate fibrotic changes in epidural fibrosis models [8,11]. The histopathologic results of our study revealed that laminectomy caused significant epidural fibrosis 4 weeks after surgery. Both topical application and systemic administration of ROS showed better fibrosis grades than the laminectomy and the spongostan groups. Almost all rats (83.4%) in the systemic ROS group demonstrated Grade 1 epidural fibrosis, so we concluded that the systemic administration of ROS yields better results than topical application.

In addition, the mean dural thicknesses of both ROS-treated groups were lower than the control and spongostan groups. In the laminectomy group, all of the specimens showed arachnoidal involvement. Furthermore, in the systemic administration group, only one specimen (16.6%) showed arachnoidal involvement. The systemic administration of ROS prevented arachnoidal involvement.

All of the results of this study suggest that both topical application and systemic administration of ROS have beneficial effects for preventing epidural fibrosis in laminectomized rats. Systemic administration of ROS revealed better results than topical application. Despite the fact that there are numerous suggested mechanisms that may underlie the preventive effects of ROS over epidural fibrosis, the most likely mechanism is the inhibition of TGF-1β and relevant pathways.

However, this study has some limitations. The number of rats in each group should be increased to yield more robust results. The dose-dependent results should also be investigated. Also, detailed biochemical analyses and ultrastructural assessments may provide more conclusive results in future studies. Our results are significant when compared with controls, but they may not be better than other drugs that are currently available. So, testing ROS with other agents that have already been proven to prevent epidural fibrosis should provide stronger results.

Conclusions

In conclusion, macroscopic and histopathologic results revealed that both topical application and systemic administration of ROS showed meaningful preventive effects over epidural fibrosis via multiple mechanisms. The results of our study provide the first experimental evidence of preventive effects of ROS over epidural fibrosis. Therefore, in light of these results, we propose that ROS may be a potential preventive agent against postlaminectomy epidural fibrosis.

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