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Original Research

The Antifibrotic Effects of Plasminogen Activator Inhibitor-1 Antagonists are Observed in Rats with Epidural Fibrosis

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Abstract

Objectives: Epidural fibrosis occurs after laminectomy as part of the local repair mechanisms. Adhesion around the nervous tissue could cause pain and disability. In the current study, we investigated the possible antifibrotic effects of the plasminogen activator inhibitor-1 (PAI-1) antagonists in a rat laminectomy model.

Methods: The rats were randomly assigned to the control, the TM5441, and the TM5484 groups (n=6 per group). In the control group, just a laminectomy was performed. In the treatment groups, intragastric administration of PAI-1 antagonists was done after skin closure. Epidural fibrosis was investigated macroscopically and histopathologically four weeks later.

Results: In the TM5441 and TM5484 groups, the macroscopic epidural fibrosis score was less than the control group ($p<0.001$ for both groups). Microscopic epidural fibrosis score was also decreased in the TM5441 and TM5484 groups ($p>0.05$ for both groups). Fibroblast cell density classification scores in the TM5441 and TM5484 groups were lower when compared to the control group ($p>0.05$ for both groups). Fibrosis thickness was lower in the TM5441 and TM5484 groups when compared to the control group ($p<0.01$ for both groups).

Conclusion: Plasminogen activator inhibitor-1 antagonists could be a treatment alternative for the prevention of epidural fibrosis.

Keywords: Epidural fibrosis, laminectomy, plasminogen activator inhibitor-1 antagonists

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Following laminectomy, epidural fibrosis (EF) is a physiological phenomenon that commonly happens during normal wound healing.[1] After surgery, excessive EF leads to recurrent complaints by stretching and compressing the nerve root.[2] Partial laminectomy is performed during lumbar disc herniation operations, and laminectomy is a commonly used surgical treatment for treating patients with spinal stenosis.[3] However, EF following laminectomy may result in back and leg discomfort that is unresponsive to therapy because of pressure on the dura mater and nerve roots.[4] Reoperating in areas with significant fibrosis is also technically difficult and risky.[5] Although research has been done to identify antifibrotic medications,[6-8] EF is still not consistently preventable or treatable. Increased extracellular matrix component deposition and reduced tissue cellularity are two mechanisms that contribute to the etiopathogenesis of EF.[9] The pathophysiology of EF is influenced by inflammation and any adhesions that develop as a result of the fibrotic process.[10]

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Plasminogen activator inhibitor-1 (PAI-1), a significant endogenous inhibitor of plasmin-mediated fibrinolysis, encourages the formation of clots after injury.[11] PAI-1 is found in adipose tissue, platelets, vascular endothelium, liver, neutrophils, astrocytes, and plasma.[12-15] Release of PAI-1 is induced by a variety of inflammatory mediators and stimuli.[16] Polymorphonuclear leukocytes and macrophages are chemoattracted to PAI-1.[17] PAI-1 antagonists have demonstrated anti-inflammatory, antiapoptotic, and neuroprotective properties in animal models of neuroinflammation.[11,18] Our study aimed to determine the antifibrotic effects of PAI-1 antagonists in the rat laminectomy-EF model.

Methods

Animals Used for Experiments

The local animal research ethics committee at the University of Health Sciences examined and approved every experimental technique utilized in this study (20.11.2020, 06.07). The European Union Council Directive (86/609/EEC) and Helsinki Declaration were adhered to in this study. In the investigation, eighteen female Sprague Dawley rats weighing 150–250 g were employed. Every rat was housed in an environment with a 12-hour light cycle, sufficient humidity, and temperatures ranging from 22 to 25°C. Rats were randomly assigned to three groups and allowed unrestricted access to food and water:

Laminectomy Group (n=6): The procedure for a laminectomy used on the participants in this group is detailed below. The laminectomy was finished without giving the individuals in this group any topical or systemic treatment after ensuring sufficient site hemostasis. Subjects were sacrificed using the procedures outlined below on the 30th day after the operation.

TM5441 Group (n=6): After laminectomy, subjects in this group were administered intragastric 5 mg/kg TM5441. The dose was adjusted according to a previous study.[19] Subjects were sacrificed using the procedures outlined below on the 30th day after the operation.

TM5484 Group (n=6): After laminectomy, subjects in this group were administered intragastric 5 mg/kg TM5484. The dose was adjusted according to a previous study.[19] Subjects were sacrificed using the procedures outlined below on the 30th day after the operation.

Surgical Procedure

Before the procedure, 20 mg/kg of cephazolin was administered intramuscularly to avoid surgical site infections. To induce anesthesia, the rats were given intraperitoneal injections of 10 mg/kg xylazine (Rompun, Bayer, Türkiye) and 50 mg/kg ketamine (Ketalar, Parke Davis, Türkiye). The animals' body temperatures were maintained at 37°C by placing them on a heating pad and inserting a rectal probe. Laminectomy was performed at the L2 level, as described previously.[20] The rats had unrestricted access to food and drink for four weeks following surgery. After receiving a 50 mg/kg intraperitoneal dose of ketamine (Ketalar, Parke Davis, Türkiye), the rats were cervically dislocated four weeks later. The first to third vertebrae were dissected to lumbar vertebrae and paravertebral structures.

Macroscopic Evaluation of Epidural Fibrosis

Before categorizing epidural fibrosis using the Rydell scale,[21] a neurological surgeon who was unfamiliar with the study groups carefully reopened the surgical sites for a macroscopic assessment. An increase in grade means more epidural fibrosis.

Histopathological Examination

To isolate the laminectomy area for histological analysis, the upper L1 to lower L3 levels of the spine were sliced axially. Hematoxylin and eosin (H&E, Shandon Harris Hematoxylin-Eosin Y -UK) and Masson trichrome (Bio-Optica special stains kit, Milan, Italy) stainings were performed. Assessments were made on the degree of any inflammation, fibrosis, scar tissue formation, and other histological alterations. Bozkurt et al.[8] proposed the parameters for the microscopic evaluation of EF in 2019. As the number of fibroblast cells increases, so does the fibroblast cell density grade.

Statistical Analysis

Data analysis was conducted using GraphPad Prism 8.0 (GraphPad Software Inc., La Jolla, CA, USA). A Tukey posthoc test was used after a one-way analysis of variance to look at the differences between the groups. A p-value of 0.05 was the cutoff point for statistical significance.

Results

Wound Healing and Complications Related to the Procedure

No deaths, deficits, infections, hematomas, erythema, or cerebrospinal fluid leaks were discovered in experimental groups.

Macroscopic Evaluation of Epidural Fibrosis

In the laminectomy group, severe epidural adhesions (n=4 grade 3 and n=2 grade 2) were observed. Mild epidural adhesions (n=2 grade 1, n=1 grade 2, n=3 grade 0) were observed in the TM5441 group and TM5484 group (n=3 grade 1, n=3 grade 0). The epidural fibrosis severity score was decreased in the TM5441 and TM5484 groups when compared with the laminectomy group ($p<0.001$ for both comparisons) (Fig. 1).

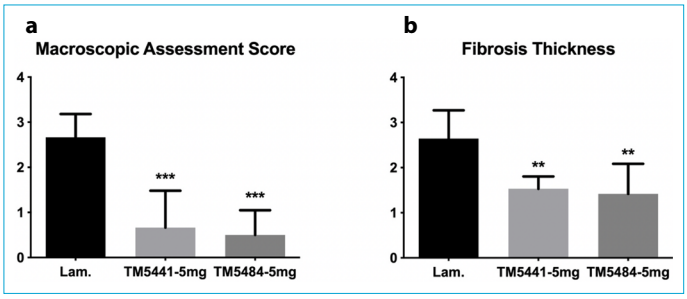

Figure 1. Macroscopic assessment of epidural fibrosis score of study groups. Data are presented as mean±SD. After laminectomy, severe epidural adhesions were observed in the laminectomy group. **(a)** Macroscopic epidural fibrosis score was decreased statistically significantly in TM5441 and TM5484 groups when compared with the laminectomy group (***: p<0.001). **(b)** Fibrosis thickness was significantly lowered in the TM5441 and TM5484 groups (**: p<0.01). L: Local; Lam: Laminectomy.

Histopathologic Assessment

Microscopic EF scores were decreased in the TM5441 and TM5484 groups when compared with the laminectomy group (p>0.05 for both comparisons). Fibroblast cell density classification scores were decreased in the TM5441 and TM5484 groups when compared with the laminectomy group (p>0.05 for both comparisons). Mean scores for fibrosis thickness were decreased in the TM5441 and TM5484 groups when compared to the laminectomy group (p<0.01 for both comparisons) (Table 1). Cartilage destruction was seen in 1 rat in the TM5484 group, and bone destruction was not seen in any rat (Fig. 2).

Discussion

During normal wound healing, fibrosis development is a physiological process that regularly takes place. Nonetheless, a variety of circumstances, both during and after laminectomy, may lead to increased fibrosis.[22] Due to oxidative stress and inflammatory mediators brought on by surgical trauma and tissue damage, inflammatory cells penetrate the surgical field.[23] Local fibroblasts develop as a result of the cytokines released by these inflammatory cells. An excessively high level of fibroblast proliferative activity and fibroblast stimulation results in the production of extracellular matrix components, including fibronectin and collagen.[24]

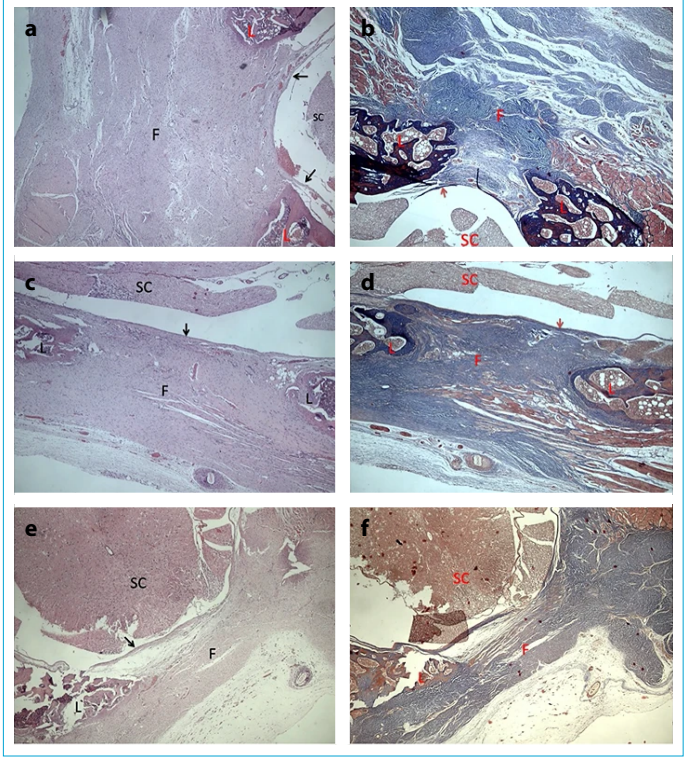

Figure 2. Representative photomicrograph of the epidural fibrosis analysis of the study groups. **(a, b)** In the control group, severe epidural fibrosis is observed (Fibrosis score: 3). L: Laminae; F: Fibrosis; SC: Spinal Cord; Arrow: Dura Mater **(a)** hematoxylin and eosin: H&E, x40. **(b)** MT: Masson trichrome, x40. C-D: In the TM5441 group, moderate epidural fibrosis is observed (Fibrosis score: 2). L: Laminae; F: Fibrosis; SC: Spinal Cord; Arrow: Dura Mater. **(c)** H&E, x40, **(d)** MT, x40. **(e, f)** In the TM5484 group, mild epidural fibrosis is observed (Fibrosis score:1). L: Laminae, F: Fibrosis, SC: Spinal Cord, Arrow: Dura Mater. **(e)** H&E, x40, **(f)** MT, x40.

Table 1. Microscopic evaluation results of experimental groups

	Control	TM5441	TM5484
Epidural fibrosis grade	Grade 2: n=3 Grade 3: n=3	Grade 3: n=2 Grade 2: n=4	Grade 1: n=1 Grade 2: n=3 Grade 3: n=2
Mean fibroblast grade	Grade 3: n=2 Grade 2: n=4	Grade 2: n=5 Grade 3: n=1	Grade 2: n=5 Grade 3: n=1
Fibrosis thickness (mm)	2.6±0.62	1.5±0.27	1.4±0.67
Cartilage destruction	none	none	n=1
Bone destruction	none	none	none
Foreign body reaction	n=3	n=3	n=0
Mild chronic inflammation	n=2	n=1	n=0

These excessively generated substances change the natural tissue structure, which causes large amounts of scar tissue to grow and compresses neurological structures.[25] The production of transforming growth factor-1 (TGF-1) is one of the key mechanisms that initiates the formation of EF.[24,26]

By squeezing the nerve root as a result of adhesive scar tissue, EF is a possible adverse effect of laminectomy that results in ongoing radicular pain and physical dysfunction.[4] Surgical removal of EF has a poor success rate and considerable complication rates.[27,28] Pharmacological and other interventions to prevent EF have been tried.[6-8,29-31] However, there is no clinical evidence for effective management of EF. Lumbar epidural steroid injection has a role in the management of chronic low back pain and radiculopathy that is seen in patients with lumbar EF.[32]

PAI-1 has a role in the fibrinolytic system and regulates vascular and tissue remodeling.[33] There is strong evidence that reduced extracellular matrix degradation causes matrix buildup and that plasminogen activator/plasmin is a major regulator of extracellular matrix breakdown.[33] Plasmin directly contributes to the disintegration of the extracellular matrix.[33] Renal and pulmonary fibrosis are two of the known diseases where increased PAI-1 levels have a crucial role in pathophysiology.[34] PAI-1 antagonists have antioxidant, anti-inflammatory, and antioxidant properties.[11,19] According to earlier research, TM5484 (a novel form of PAI-1 antagonist) inhibits microglia activation and macrophage migration in an experimental multiple sclerosis model.[18]

Macroscopic assessment of EF showed that both PAI-1 antagonists administration reduced EF formation. Moreover, fibrosis thickness scores were significantly lowered in PAI-1 antagonist groups. Since PAI-1 antagonists have been demonstrated to reduce TGF-1, in addition to their well-known antioxidant, anti-inflammatory, and anti-apoptotic effects, this study showed that PAI-1 antagonists may also have a therapeutic role for EF in a rat laminectomy model.

There are some limitations to this study. Due to the small number of subjects in each group, we may draw fewer conclusions. Increasing the sample size could provide more robust findings. Because biochemical tests were not conducted, our conclusions were likewise more limited. Including biochemical analyses, such as cytokine levels, inflammatory markers, or matrix metalloproteinases, would provide a better understanding of the antifibrotic mechanisms. The study does not evaluate the long-term efficacy of PAI-1 antagonists, leaving the durability of the treatment's effects uncertain. Introducing groups with varying doses of PAI-1 antagonists could help elucidate dose-dependent effects.

Conclusion

On a macroscopic and microscopic level, the PAI-1 antagonist, which is well known for its antioxidant and anti-inflammatory capabilities, inhibited the synthesis of EF. The outcomes of our investigation offer the first experimental proof of the EF-protecting abilities of PAI-1 antagonists.

Disclosures

Ethics Committee Approval: The study was approved by the University of Health Sciences Türkiye Hamidiye Animal Research Local Ethics Committee (Date: 20.11.2020, No: 06.07).

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Use of AI for Writing Assistance: The authors declared that no text or image produced by the AI was used in this work.

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