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The Effect of Halofuginone Use on Epidural Fibrosis After Spinal Surgery: An Animal Experiment

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ABSTRACT

AIM: To investigate the effectiveness of local halofuginone application for spinal epidural fibrosis (EF) after lumbar laminectomy in rats.

MATERIAL and METHODS: Forty rats were equally divided into four groups (Groups I–IV; 10 rats in each group), and lumbar laminectomy was performed under general anesthesia. After laminectomy, Group I received saline (NaCl 0.9%) locally (control), Group II received spongostan, Group III received 0.5 mL of halofuginone-impregnated spongostan, and Group IV received 0.5 mL of halofuginone. Spongostan was used to prolong the exposure period of halofuginone. All rats were sacrificed after four weeks and evaluated according to histopathological criteria. A p-value of <0.05 was considered statistically significant.

RESULTS: Fibrosis was significantly lower in Group IV than in Group I ($p<0.05$). There was no significant difference in fibrosis between Group II/III and Group I. It was observed that spongostan increased fibrosis.

CONCLUSION: Halofuginone helps prevent EF after spinal surgery. However, further clinical and experimental studies are needed to assess its safety in humans.

KEYWORDS: Dura, Halofuginone, Fibrosis, Spinal surgery, Spinal cord, Rat

ABBREVIATIONS: EF: Epidural fibrosis, **IL:** Interleukin, **MCP-1:** Monocyte chemoattractant protein-1, **MIP-1β:** Macrophage inflammatory protein-1 beta, **PDGF:** Platelet-derived growth factor, **PPARs:** Peroxisome proliferator-activated receptors, **TGF-β:** transforming growth factor beta, **VEGF:** Vascular endothelial growth factor

INTRODUCTION

Epidural fibrosis (EF) occurs naturally after surgical interventions, such as laminectomy and hemilaminectomy, which are performed to treat diseases that cause spinal cord or nerve root compression. EF can cause low back pain that might occur following these procedures (6,10). Many experimental and clinical strategies for fibrosis prevention have been investigated, but no successful treatment has yet been implemented into standard clinical practice (1,13).

Halofuginone is an analog of febrifugine, an alkaloid obtained from the plant *Dichroa febrifuga* "Lour" (18). Transforming growth factor beta (TGF-β) regulates fibroblast function (4),

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and TGF-β stimulation induces fibroblast activation, causing a transition to myofibroblasts, which are critical effector cells in fibrotic conditions. Halofuginone use leads to a decrease in the differentiation of fibroblasts, a reduction in extracellular matrix protein levels, and the inhibition of fibrosis and tumor growth by inhibiting TGF-β-dependent Smad3 phosphorylation (19). The present study aimed to investigate the effectiveness of local halofuginone application for spinal EF after lumbar laminectomy in rats.

MATERIAL and METHODS

The institutional review board of Istanbul University, Istanbul Faculty of Medicine, Turkey reviewed and approved (26/10/2017 - 402443) the study protocols and steps.

Experimental Groups

The study included 40 adult male Sprague–Dawley rats (weighing 250–350 grams) bred at Istanbul University's Aziz Sancar Experimental Medicine Research Institute. The rats were fed regular rat chow and tap water before the experiment and were kept in their typical surroundings with a 12-hour dark/12-hour light cycle.

The experimental rats were randomly divided into the following four groups (10 animals in each group): Group I (control), which included rats that were administered no therapy; Group II, which included rats that were administered spongostan; Group III, which included rats that were administered halofuginone-impregnated spongostan; and Group IV, which included rats that were administered halofuginone. Spongostan was used to prolong the exposure period of halofuginone.

Anesthesia

All rats in our study fasted the day before surgery. For the surgical procedure, general anesthesia was induced intraperitoneally with 60 mg/kg of ketamine hydrochloride (Ketalar; Parke Davis, Eczacibasi, Istanbul) and 5 mg/kg of xylazine hydrochloride (Rompun; Bayer, Leverkusen, Germany). If an additional dose was needed, anesthesia was continued with ketamine at the rate of 20% of the initial dose.

Surgical Procedure

For prophylaxis, a single dose of 50 mg/kg of cefazolin sodium (Sefazol; Mustafa Nevzat, Istanbul, Turkey) was administered intraperitoneally 30 minutes before surgery. The rats were fixed in the prone position, and then, the lower half of the dorsal region was shaved for surgical preparation. The surgical field was rinsed with povidone-iodine scrub (Medica brush; 4% chlorhexidine soap; Medica BV, Oss, The Netherlands) and disinfected by staining with povidone-iodine solution (Poviod; 10% polyvinylpyrrolidone complex; Saba, Istanbul, Turkey). The surgical area was covered with a sterile drape. A skin incision was made along the midline, stretching from L1 to the sacrum, by using the sacroiliac crest as a landmark. The paraspinal muscles were stripped by blunt dissection, and the lumbar vertebrae were located. Following the blunt dissection, a three-level (L3–L4–L5) laminectomy was performed using a curved hemostat under a microscope. After the dura was exposed, Group I received saline (NaCl 0.9%) locally, Group II received spongostan, Group III received 0.5 mL of halofuginone-impregnated spongostan, and Group IV received 0.5 mL of halofuginone. After completing hemostasis, the muscle was closed using 4-0 Vicryl and skin staple sutures. The wound was dressed with povidone-iodine when the surgical procedure was completed. The study excluded rats with a dural tear or a nerve root injury during surgery.

Rats were sacrificed using intraperitoneal pentobarbital at 120 mg/kg after 4 weeks of follow-up. The surgical area was evaluated for infection, and the vertebral column was removed as a block, including the laminectomy areas. Biopsy samples were submitted to the Pathology Department of Istanbul Medical Faculty at Istanbul University in sterile numbered containers filled with alcohol–formaldehyde–acetic acid.

Pathological Examination

The specimens were decalcified (Decal; ½ 10% formic acid + ½ 8% HCL) for 2 days in the Pathology Department. After the decalcification procedure, the specimens were examined macroscopically by a pathologist. Several samples were collected, including those from the laminectomy site. These samples were subjected to tissue follow-up in an autotechnicon. Paraffin-embedded blocks were obtained. For histopathological examination, serial sections were obtained from each block. The samples were stained with hematoxylin and eosin and Masson's trichrome stains. The prepared cells were examined using a light microscope. The amount of scar tissue in the produced slides was assessed histopathologically. The amount of EF was evaluated based on the criteria set by He et al. (Table I) (9).

Statistical Analysis

The distribution of quantitative variables was assessed with normality measures of kurtosis and skewness. The median (range) was used to depict quantitative data, and the Mann–Whitney *U* test was used to analyze group differences. The qualitative data were presented using frequency and percentage. A chi-square test was used to compare all qualitative data. All statistical analyses were performed using IBM SPSS Version 20.0 (IBM Corp., Armonk, NY). A p-value of <0.05 was considered statistically significant.

RESULTS

In Group I, there was macroscopic evidence of diffuse fibrosis in the epidural area at the laminectomy site, and all rats had grade 2–3 fibrosis (Figure 1). In Group II, significant fibrosis was found both macroscopically and microscopically, and 80% of the rats had grade 2–3 fibrosis (Figure 2). In Group III, fibrosis and adhesions were prevalent (Figure 3). In Group IV, minimal scar tissue and adhesions were observed in most of the rats, and grade 1 fibrosis was found in 60% of the rats (Figure 4). Table II shows the fibrosis rates in the study groups. In Group I, six rats had grade 2 fibrosis and four rats had grade 3 fibrosis, but in Group II, two rats had grade 1 fibrosis, four had grade 2 fibrosis, and four had grade 3 fibrosis. Although Group II had less fibrosis macroscopically, there was no statistically significant difference in the rate of grade 1–2 fibrosis between the two groups (p=0.684) (Table II).

Table I: The Histological Evaluation Criteria

Grade 0	No fibrosis affecting the dura mater
Grade 1	Only thin fibrous bands exist between fibrous tissue and dura mater
Grade 2	Continuous adherence is observed in less than 2/3 of the laminectomy defect
Grade 3	There is adhesion of fibrous tissue in greater than 2/3 of the laminectomy defect and/or the fibrous tissue extends up to the nerve roots

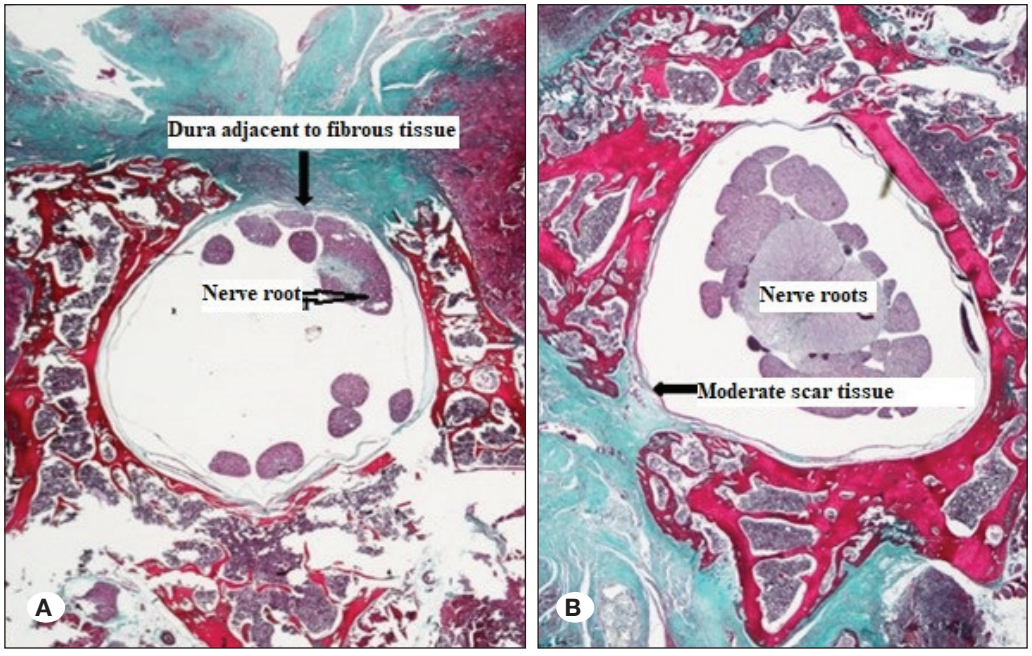

Figure 1: Grade 3 fibrosis, in which the dura is surrounded by advanced fibrous tissues, and grade 2 fibrosis, in which there are gaps between the dura and fibrous tissues (Masson's trichrome staining; magnification, ×10).

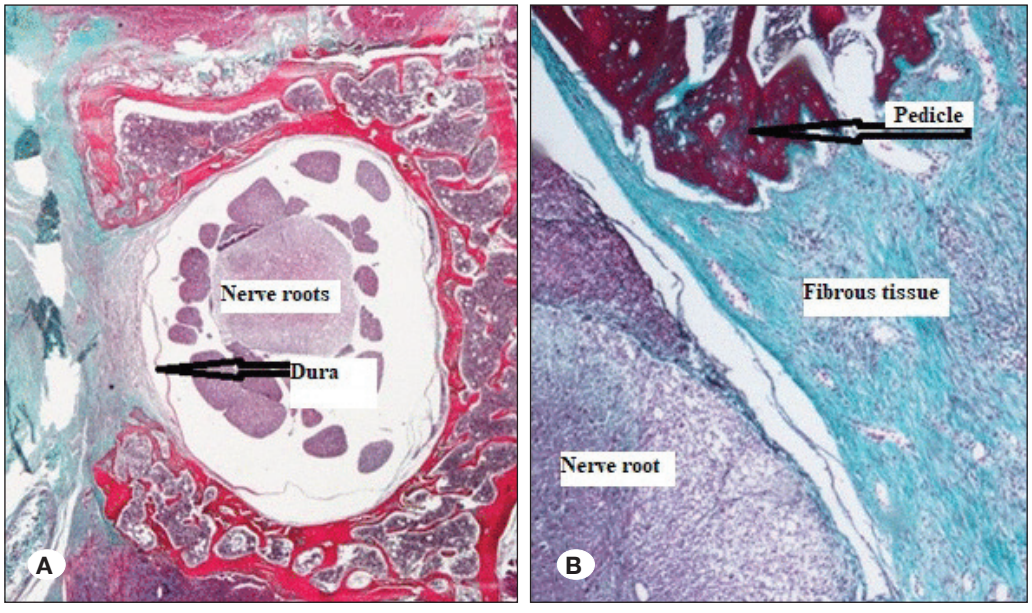

Figure 2: Grade 2 fibrosis (Masson's trichrome [MT] staining; magnification, ×10) with distinct dura and fibrous tissue, and grade 3 fibrosis (MT staining; magnification, ×40), in which fibrous tissues adhere to the dura and extend below the pedicle.

Table II: Comparison of the Prevalence of Epidural Fibrosis Between Groups

	Grade 1 fibrosis Number (%)	Grade 2 fibrosis Number (%)	Grade 3 fibrosis Number (%)
Group I (C)	0 (0)	6 (60)	4 (40)
Group II (S)	2 (20)	4 (40)	4 (40)
Group III (H + S)	0 (0)	7 (70)	3 (30)
Group IV (H)	6 (60)	2 (20)	2 (20)

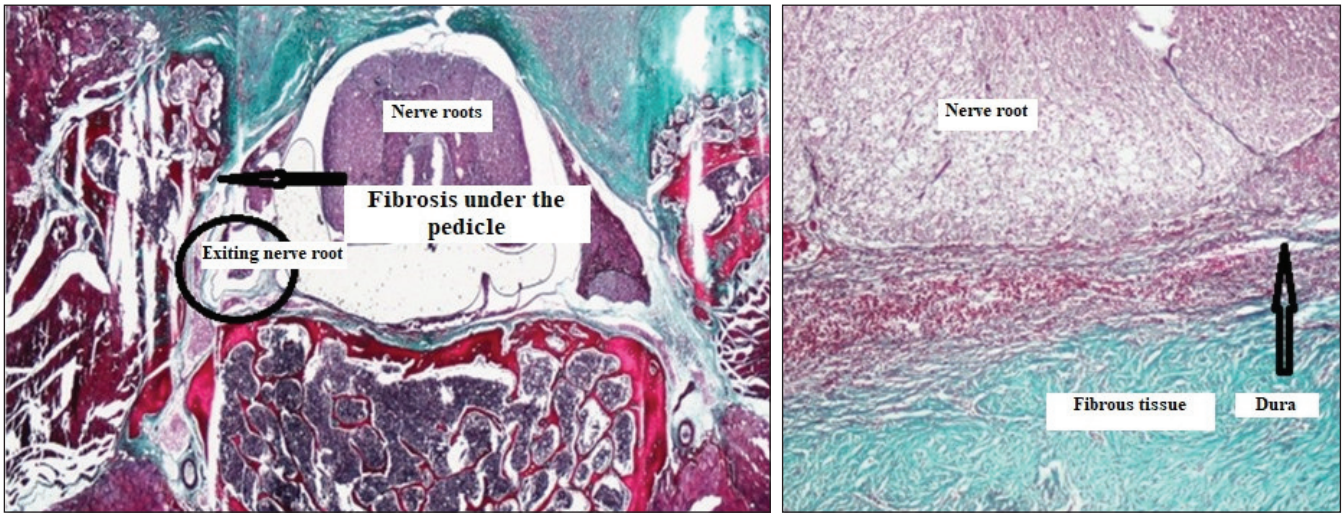

Figure 3: Grade 3 epidural fibrosis with extensive scar tissue extending to the nerve root. The scar tissue that has invaded the dura adheres to the dura in the medulla, and there is extensive thickening in the dura (Masson's trichrome staining; magnification, ×10 and ×40).

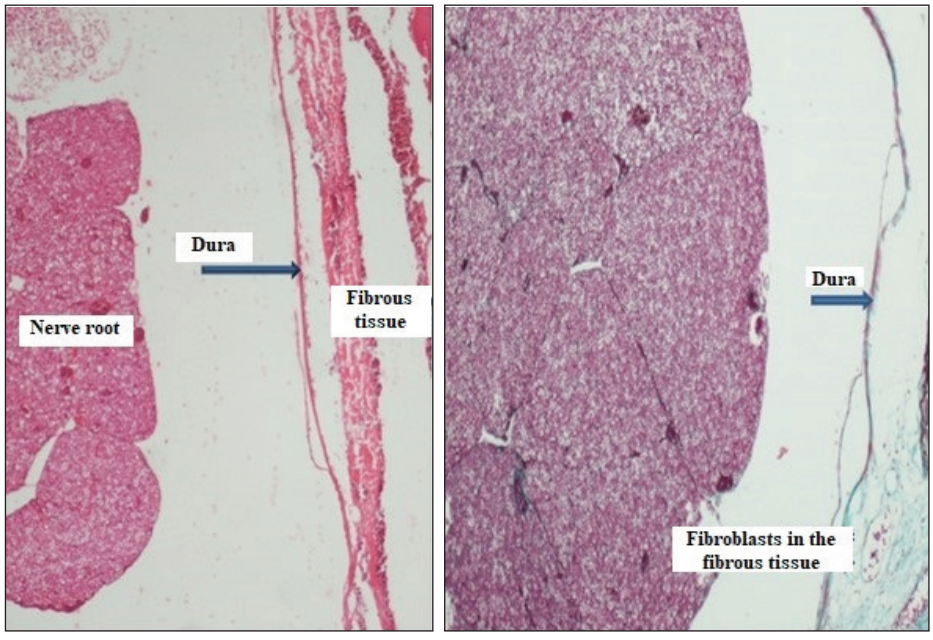

Figure 4: Grade 1 fibrosis with minimal scar tissue, reduced number of fibroblasts, and free dura.

DISCUSSION

EF after spinal surgery can cause persistent back and leg pain, and severe complications, which can necessitate reoperation. The resulting fibrous connective tissue can compress the dura mater and nerve root. Therefore, effective prevention of EF is critical for surgical success and pain management (6,8).

Repeated procedures to relieve the secondary pressure caused by EF may increase fibrosis. It is preferable to prevent this in the first operation (11). The main component of scar tissue is collagen. According to Barbera et al., the primary substance originating from the spinal cord muscles and filling the laminectomy defect is collagen, and the amount of collagen is proportionate to the extent of scar tissue (3). Similar to previous studies, our study showed that dense fibrosis formed after laminectomy in 40% of control rats, which surrounded the bones, and dura mater.

The idea of separating the dura from tissues undergoing postoperative healing has been proposed in theory. Ozer et al. separated patients who underwent surgery for lumbar disk herniation into two groups (with and without ligamentum flavum preservation) to assess the protection ability of the ligamentum flavum. They reported that at a 6-month follow-up, EF formation was lower with ligamentum flavum preservation than without ligamentum flavum preservation (17). Autogenous barriers are practical materials that are currently available. Although free fat grafts constitute a good barrier, they do not sufficiently reduce fibrosis and can even cause cauda equina syndrome in rare cases (12). The use of antiadhesion materials following disk herniation surgery has been reported to help prevent the development of EF. The effect of such a material would indirectly support the theory that peridural scarring causes complications. In a study of 396 patients by Fransen et al., it was revealed that coating the dura with carboxymethylcellulose/polyethylene oxide reduced pain and symptoms, and even reduced fibrosis in patients who underwent reoperation (7).

The key regulators of fibrosis are cytokines (interleukin [IL]-13, IL-21, and TGF-β1), chemokines (monocyte chemoattractant protein-1 [MCP-1] and macrophage inflammatory protein-1 beta [MIP-1β]), angiogenic factors (vascular endothelial growth factor [VEGF]), growth factors (platelet-derived growth factor [PDGF]), peroxisome proliferator-activated receptors (PPARs), acute phase proteins, caspases, and components of the renin–angiotensin–aldosterone system. These regulators have been identified as potential targets for antifibrotic therapy (4, 19). An agent that is expected to inhibit the production of scar tissue can be applied locally or orally without harming the patient. Halofuginone, which was utilized in our study, is an effective agent in the TGF-β pathway. The TGF-β pathway and type I collagen deposition have been identified as effective mechanisms of fibrosis in both experimental and clinical research (4,19).

Since the early 1990s, halofuginone has been utilized in numerous domains of human medicine and is recognized to have no evident toxic effects. This agent has been proposed as a new therapy option in experimental studies on adhesion, fibrosis, sclerosis, cirrhosis, and stenotic diseases of luminal organs. It suppresses collagen synthesis by influencing immune system cells and cytokine synthesis (14). In an in vitro study, it was shown that up to 10 ng/mL of halofuginone was tolerated and had an effect on human corneal fibroblasts. Moreover, halofuginone significantly reduced TGF-β-induced expression of fibrotic markers, alpha-smooth muscle actin (α-SMA), fibronectin, and type I collagen at this concentration. Furthermore, halofuginone treatment resulted in a decrease in the expression of Smad3 in a dose- and time-dependent manner (21).

In a Duchenne muscular dystrophy mouse model, the group treated with halofuginone for 10 weeks had better motor coordination and less inflammation, degeneration, and fibrosis in the diaphragm compared with the findings in the control group. There have been no measurements of the agent's metabolites in plasma or tissues, and it has only been shown to be toxic at extremely high doses (20). Furthermore, halofuginone has been demonstrated to prevent extracellular matrix accumulation; suppress fibroblast function, angiogenesis, and neovascularization; and reduce postoperative adhesions (16).

Halofuginone inhibits TGF-β-dependent Smad3 phosphorylation. It causes a decrease in the differentiation of fibroblasts and a reduction in extracellular matrix protein levels, thus inhibiting fibrosis and tumor growth (15, 21). According to our study, when halofuginone was used locally, it greatly reduced fibrosis. However, it was shown that spongostan, which was used to extend the exposure period of halofuginone, increased fibrosis, although the finding was not statistically significant. Based on this finding, another substance should be utilized to prolong the exposure period of halofuginone locally. On the other hand, orally administered halofuginone for fibrosis has been reported to be effectively absorbed and tolerated by the gastrointestinal tract (5). A new study may also look into the effects of oral halofuginone administration on EF.

Aslan et al. showed the effect of halofuginone at the laminectomy site. Their study included 21 rats divided into the following groups: only laminectomy group (control), laminectomy with hydrogel placement group, and laminectomy with both hydrogel and halofuginone placement group. They identified intense fibrosis in the control group, a membranous barrier that prevented fibrosis in the hydrogel group, and low fibrosis levels in the hydrogel and halofuginone group (2). In our study, although spongostan was thought to provide a mechanical barrier and increase the exposure period of halofuginone, spongostan increased fibrosis at the laminectomy site. The findings of the study show that halofuginone might be effectively used in humans. Clinical trials should be performed to assess its safety after spinal surgery in humans.

CONCLUSION

Based on the findings of our study, halofuginone could be utilized to prevent EF after spinal surgery. The ability to safely use this agent in the human body is crucial for its general use. Our findings will inspire other scientists to conduct large clinical trials and develop more complex treatment strategies using this agent.

AUTHORSHIP CONTRIBUTION

Study conception and design: MZY, BP, TCU, ID

Data collection: MZY, BP, DD

Analysis and interpretation of results: CIG, DD, ES, TCU, ID

Draft manuscript preparation: CIG, DD

Critical revision of the article: YA, AA, PAS, AS, ANI

All authors (MZY, BP, TCU, ID, CIG, DD, ES, YA, AA, PAS, AS, ANI) reviewed the results and approved the final version of the manuscript.

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