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Basic Science

Effects of hemostatic polysaccharide agent on epidural fibrosis formation after lumbar laminectomy in rats

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Abstract

BACKGROUND CONTEXT: Epidural fibrosis is a common adverse outcome of spinal surgery that can compress the dural sac and nerve root. Local hemostatic agents have many indications in numerous types of spinal surgery. As these agents may behave as foreign bodies, inducing inflammation and delaying regeneration, they could enhance the risk of epidural fibrosis.
PURPOSE: We evaluated the effects of hemostatic polysaccharide on epidural fibrosis development in laminectomized rats.
STUDY DESIGN: This is a randomized controlled trial.
OUTCOME MEASURES: One month after surgery, tissues were histopathologically examined. Spinal tissue surrounding the laminectomy site was cut with a microtome and stained with hematoxylin and eosin and Masson trichrome. Slides were evaluated by a pathologist in a blinded fashion. The extent of epidural fibrosis, fibroblast cell density, cartilage, and bone regeneration was evaluated.
METHODS: Rats were randomly assigned to receive sham surgery, laminectomy, or laminectomy with hemostatic polysaccharide (seven rats per group). Sham surgery that consisted of a skin incision was performed without laminectomy. Laminectomy was performed at the L1 and L2 vertebrae. In the experimental group, the polysaccharide hemostatic material, HaemoCer was placed in the laminectomy area.
RESULTS: The proportion of rats with epidural fibrosis in laminectomized mice (both with and without hemostatic material) was higher than in sham-operated rats ($p<.01$). There was no difference in fibrosis between the two groups of laminectomized rats ($p>.05$).
CONCLUSIONS: Our study indicates that hemostatic polysaccharide does not enhance epidural fibrosis following laminectomy in rodents, suggesting that absorbable polysaccharides may be appropriate for use in hemostasis during spinal surgery.

Keywords: Epidural fibrosis; Hemostatic polysaccharide; Laminectomy; Rat; Spinal surgery; Hemostasis

Introduction

Epidural fibrosis (EF), a replacement of normal epidural fat with postoperative fibrotic tissue, which is capable of binding the dura and nerve roots to the surrounding structures anteriorly and posteriorly [1–3], is an unwanted consequence of spinal surgery. Such fibrotic deposits can compress neural tissue, and thus are thought to underlie poor outcomes in spinal surgery [1].

Preliminary studies on the etiopathology of EF showed that EF originates from a "laminectomy membrane" that comprised the bleeding surface of the deep layer of the posterior paravertebral muscles [4,5]. This laminectomy membrane covers the defect created by the bone resection in an attempt to replace the empty space. Fibrous tissue aims to reconstitute the removed lamina and extends within the neural canal [4,5]. Subsequent research on the biochemical mechanism of

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EF showed that disc herniation activates the arachidonic acid cascade. This activation results in the production of prostaglandins E1 and E2 and leukotriene B, substances that contribute to an inflammatory process [6]. This reaction persists after discectomy [6]. The additional influencing factors of EF development can be patients' humoral or immunologic factors, size and duration of the disc herniation, intraoperative trauma (amount and technique of soft tissue and disc dissection), perioperative steroid treatment, and postoperative mobilization and activity levels [7].

Epidural fibrosis can cause pain in the back or lower extremities for up to 6 months postsurgery [8]. Several surgical techniques have been suggested to prevent or limit postoperative scarring [9,10], some of which have been experimentally tested [8–10]. Similarly, pharmacologic agents and interventions, such as rosuvastatin, temozolomide, poly thermogel, and hyperbaric oxygen, have been used to prevent EF [1,2,11].

Inadequate hemostasis is the most important risk factor in spine surgery. Spinal surgeons routinely use bipolar electrocautery and slight manual pressure or topical hemostatic agents. Hemostatic agents should have strong hemostatic efficacy to promote healing without causing tissue reaction or inflammation; other desirable characteristics are biodegradability, cost-effectiveness, ease of use, and non-interference with imaging [12,13]. Hemostatic polysaccharide is manufactured from a purified plant-based polysaccharide, formulated into spherical particles (40–150 μm) with a large microporous surface. These particles reduce bleeding by rapid dehydration and subsequent concentration of red blood cells, platelets, and serum proteins to produce a gelled matrix. The surface of this gelled matrix then stimulates the clotting cascade; platelet activation and fibrin deposition produce a clot that limits further bleeding. Complete absorption is achieved within approximately 2 days, degrading by endogenous alpha-amylase [14,15].

Hemostatic agents have been linked to the formation of granulomas, which can cause postoperative pain. Residual hemostatic agent may behave as a foreign body, inducing inflammation and even delaying bone growth [16]. Therefore, we evaluated the effects of hemostatic polysaccharide on EF development following laminectomy in rats.

Materials and methods

Experimental design and animal care

The experimental protocol was approval by the Animal Care and Use Committee at Marmara University School of Medicine. Twenty-one male Sprague-Dawley rats weighing approximately 250 to 300 g were housed, with one animal per cage, at the Animal Experimental Research Centre (DEHAMER) of Marmara University. The animals were fed a standard rodent chow diet and water ad libitum, and were kept at a constant temperature (22°C) on a 12:12 h light : dark cycle.

Table 1
Grading of epidural fibrosis

Grade	Definition
1	The dura mater was free of scar tissue.
2	Only thin fibrous bands between scar tissue and dura mater were observed.
3	Continuous adherence was observed but was less than two-thirds of laminectomy defect.
4	Scar tissue adherence was large, more than two-thirds of laminectomy defect, or extended to the nerve roots.

Surgical procedure

Rats were randomly allocated into three groups (sham surgery, laminectomy, or laminectomy with hemostatic polysaccharide [HaemoCer, Bayreuth, Germany]; seven rats per group) using sealed envelopes, selected by a physician. Rats were anesthetized by intraperitoneal injection of ketamine hydrochloride (90 mg/kg, Ketalar, Pfizer, Istanbul, Turkey) and xylazine hydrochloride (10 mg/kg, Rompun 2%, Bayer, Istanbul, Turkey), and placed on an operating board in a prone position. The dorsal hair of each rat was shaved, and the surgical field was disinfected with povidone-iodine and draped with sterile towels. A dorsal midline incision was made between the twelfth thoracic and third lumbar vertebrae. After paravertebral dissection of the muscle, laminectomy was performed on the first and second lumbar vertebrae. In the sham surgery group, only skin incision and muscle dissection were performed. For postoperative analgesia, all of the rats were given ketorolac (50 mg/kg, intraperitoneal) for 5 days.

Histopathologic analyses

One month after surgery, spinal blocks were dissected and preliminarily fixed and preserved in 10% buffered formalin, and then placed in fixation and decalcification solution (BioCal C, code RRDC3/G, composition: EDTA <1%, potassium sodium tartrate <1%, sodium tartrate <1%, and hydrochloric acid <1%; Biostain, Traralgon, Australia) for 36 h. Three thick horizontal sections (3 mm) were collected from the laminectomy site in each spinal tissue sample. After processing (Leica ASP300 S, Wetzlar, Germany), sections were embedded in paraffin, and 4-μm serial sections were cut with a microtome and stained with hematoxylin and eosin (Shandon Harris, Leicestershire, UK) and Masson trichrome (Bio-Optica Kit, Milan, Italy). Sections were evaluated by the same pathologist using an Olympus CX41 RF trinocular light microscopy (Tokyo, Japan). The extent of EF at the laminectomy scar (Table 1) was determined according to the criteria defined by He et al. [17].

Table 2
Grading of fibroblast cell density

Grade	Definition
1	Less than 100 fibroblast cells per 400× field (+)
2	100–150 fibroblast cells per ×400 field (++)
3	More than 150 fibroblast cells per ×400 field (+++)