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 < 0.01). There was no difference in fibrosis between the two groups of laminectomized rats (p > 0.05).

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

Epidural fibrosis can cause pain in the back or lower ex-
tremities for up to 6 months postsurgery [8]. Several surgical
techniques have been suggested to prevent or limit postop-
erative scarring [9,10], some of which have been
experimentally tested [8–10]. Similarly, pharmacologic agents
and interventions, such as rosuvastatin, temozolomide, poly
thermol, 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 elect-
rocautery and slight manual pressure or topical hemostatic
agents. Hemostatic agents should have strong hemostatic ef-
cicacy to promote healing without causing tissue reaction or
inflammation; other desirable characteristics are biodegrad-
ability, cost-effectiveness, ease of use, and non-interference
with imaging [12,13]. Hemostatic polysaccharide is manu-
factured from a purified plant-based polysaccharide, formulated
into spherical particles (40–150 μm) with a large microporous
surface. These particles reduce bleeding by rapid dehydra-
lation 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; plate-
let activation and fibrin deposition produce a clot that limits
further bleeding. Complete absorption is achieved within ap-
proximately 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 he-
mostatic agent may behave as a foreign body, inducing
inflammation and even delaying bone growth [16]. There-
fore, 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 Med-
icine. 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.

Surgical procedure

Rats were randomly allocated into three groups (sham
surgery, laminectomy, or laminectomy with hemostatic poly-
saccharide [HaemoCer, Bayreuth, Germany]; seven rats per
group) using sealed envelopes, selected by a physician. Rats
were anesthetized by intraperitoneal injection of ketamine hy-
drocortisone (90 mg/kg, Ketalar, Pfizer, Istanbul, Turkey) and
xylazine hydrochloride (10 mg/kg, Rompun 2%, Bayer, Is-
tanbul, Turkey), and placed on an operating board in a prone
position. The dorsal hair of each rat was shaved, and the sur-
geo 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 per-
formed 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 laminec-
tomy site in each spinal tissue sample. After processing (Leica
ASP300 S, Wetzlar, Germany), sections were embedded in par-
affin, and 4-μm serial sections were cut with a microtome and
stained with hematoxylin and eosin (Shandon Harris, Leices-
tershire, 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>
<thead>
<tr>
<th>Table 1</th>
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<tr>
<td>Grade</td>
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<td>1</td>
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<td>2</td>
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<tr>
<td>3</td>
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<td>4</td>
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<th>Table 2</th>
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<tr>
<td>Grade</td>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
</tr>
</tbody>
</table>
Fibroblast cell density grades are presented in Table 2. Cartilage and bone regeneration at the laminectomy site was absent (score of 0) or mild (score of 1)[17]. These data were used to calculate the rate of bone and cartilage regeneration.

**Statistics**

Statistical analyses were performed using Number Cruncher Statistical System (NCSS) 2007 and Power Analysis and Sample Size (PASS) 2008 software (NCSS LLC, Kaysville, UT, USA). Interim power has been performed after three animals included in each group analysis showed that seven animals in each group are enough to achieve 80% power. Descriptive statistics of data were presented as median, range, frequency, and rate. Assumptions of statistical tests were checked. We used Shapiro-Wilk test to evaluate if data were normally distributed. The Mann-Whitney U test and Kruskal-Wallis analysis of variance were used for comparison of two or three independent groups with non-normal distribution, respectively. The Fisher-Freeman-Halton test was used for comparisons of cartilage and bone regeneration. For all of the statistical tests, values of $p < 0.05$ were considered statistically significant.

**Results**

Epidural fibrosis was significantly greater in both groups of laminectomized rats than in sham-operated rats ($p = 0.001$). Fibrosis did not differ between the rats treated with hemostatic polysaccharide and those laminectomized without hemostasis ($p = 0.298$) (Figs. 1–3). Fibroblast density was also significantly greater in both groups of laminectomized rats than in sham-operated rats ($p < 0.01$). The ratio of rats with fibroblast density scored as “++” or “+++” was higher in laminectomized than in sham-operated rats ($p = 0.001$). This measure also did not differ according to hemostatic treatment ($p = 0.591$).

The proportion of rats with cartilage regeneration was higher in both groups of laminectomized rats than in sham-operated rats ($p = 0.007$ and $p = 0.023$ for plain laminectomy and
laminectomy with hemostatic polysaccharide, respectively). Cartilage regeneration did not differ between the rats treated with hemostatic polysaccharide and those laminectomized without hemostasis ($p = .591$). Bone regeneration did not differ between groups ($p > .05$). The rate of bone regeneration among all of the laminectomized rats was 43% (Table 3).

### Discussion

The rate of poor outcomes in spinal surgery is 5 to 30%, and one potential cause of poor outcomes in spinal surgery is the formation of epidural scar tissue [6,7,9,10]. Moreover, EF is the most problematic complication reported by surgeons for spinal procedures [10]. The degree of postlaminectomy EF has been shown to correlate with the extent of lamina dissection [18,19]. Importantly, reoperation is generally not advised because of the risk of dura tearing, excessive bleeding, and nerve root injury [10].

The development of EF involves several steps. An acute inflammatory reaction after surgery is followed by ischemia, local toxicity, microvascular changes, and hematoma. These processes support the formation of fibrotic scar tissue in the epidural and arachnoid spaces [8,9,17,18,20]. This scar formation can cause back pain, paresthesia, and neurologic deficit, extending hospitalization, increasing treatment cost, and causing patient reliance on analgesics [3,9,16].

Several approaches have been investigated to prevent and reduce scar formation, and these include the use of steroids, corticosteroids, and growth factors. These agents work by inhibiting fibroblast proliferation, collagen synthesis, and matrix formation.

### Table 3

Rates of various grades of epidural fibrosis, fibroblast density, and cartilage and bone regeneration among rats that are sham-operated, laminectomized, or laminectomized with hemostatic polysaccharide

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Group C</th>
<th>Group L</th>
<th>Group P</th>
<th>p Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidural fibrosis, N</td>
<td>None</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+++</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Median (Q1–Q3)</td>
<td>2 (0.5–3)</td>
<td>0 (0–1)</td>
<td>2 (2–3)</td>
<td>3 (2–3)</td>
<td>$p &lt; .001$</td>
</tr>
<tr>
<td>Fibroblast density, N</td>
<td>None</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>9</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+++</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Median (Q1–Q3)</td>
<td>2 (0.5–2.5)</td>
<td>0 (0–1)</td>
<td>2 (2–3)</td>
<td>2 (2–3)</td>
<td>$p &lt; .001$</td>
</tr>
<tr>
<td>Cartilage regeneration, N (%)</td>
<td>(+)</td>
<td>12 (57.1)</td>
<td>7 (100)</td>
<td>2 (28.6)</td>
<td>3 (42.9)</td>
</tr>
<tr>
<td></td>
<td>(−)</td>
<td>9 (42.9)</td>
<td>5 (71.4)</td>
<td>4 (57.1)</td>
<td>$p &lt; .023$</td>
</tr>
<tr>
<td>Bone regeneration, N (%)</td>
<td>(+)</td>
<td>15 (71.4)</td>
<td>7 (100)</td>
<td>4 (57.1)</td>
<td>4 (57.1)</td>
</tr>
<tr>
<td></td>
<td>(−)</td>
<td>6 (28.6)</td>
<td>3 (42.9)</td>
<td>3 (42.9)</td>
<td>$p &lt; .171$</td>
</tr>
</tbody>
</table>

CI, confidence intervals.

Q1: 25th percentile, 1st quartile; Q3: 75th percentile, 3rd quartile.

* $p < .05$; ** $p < .01$.

† Kruskal-Wallis test.

‡ Fisher-Freeman-Halton test.

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non-steroidal anti-inflammatory drugs, and implanting materials such as free or pedicle fat grafts, synthetic membranes, hemostatic sponges, and anti-adhesion barrier gels [9,10,17]. However, these agents only show limited efficacy [10].

Bleeding control is the most important factor in the success of surgery in the central nervous system, so hemostatic agents are frequently used. The most common hemostatic agents are gelatin sponge (Gelfoam, Pfizer, New York, NY, USA), oxidized cellulose (SURGICEL, Ethicon, Inc, Somerville, NJ, USA), microfibrillar collagen (Avitene, Alcon, Inc, Humacao, Puerto Rico), and gelatin matrix thrombin sealants (FLOSEAL, Baxter Healthcare Corporation, Deerfield, IL, USA) [4,13,21,22]. Hemostatic agents sometimes appear on magnetic resonance imaging as tumor-like masses [21]. However, Tschan et al. [14] found no tumor-mimicking contrast enhancement on magnetic resonance imaging following hemostatic polysaccharide administration in brain tumor surgery. Oxidized cellulose and gelatin sponges have been shown to cause late tissue reactions, perilesional edema, and granuloma [23,24]. Similarly, residual oxidized cellulose, microfibrillar collagen, and gelatin matrix thrombin sealants can cause granuloma formation [25]. To the best of our knowledge, no studies have determined if hemostatic polysaccharide affects the development of EF following laminectomy.

In the present study, the hemostatic plant-based polysaccharide HaemoCer was used to seal the laminectomy site. Histopathology showed that, although laminectomy promoted EF and increased the density of fibroblasts and cartilage regeneration, application of HaemoCer did not increase EF.

Various other hemostatic agents have been investigated using methodologies similar to the current study [26–28]. They evaluated TachoComb (a fibrin-coated collagen fleece) and FLOSEAL (gelatin containing thrombin-based hemostatic agent). TachoComb’s and FLOSEAL’s EF formation extension and severity were found significantly lower than the control group [26–28]. Also, Le et al. [26] found that TachoComb causes less EF than SPONGOSTAN (absorbable hemostatic gelatin sponge) and TABOTAMP (oxidized regenerated cellulose). We found no statistical differences between laminectomy and laminectomy with HaemoCer group. These results suggest that the hemostatic agent’s mechanism of action and effectiveness might explain the differences between the present study and studies on other hemostatic agents. Research about the effect of hemostatic agent on EF has given us insight to evaluate comparative study on these hemostatic agents in the future.

Hemostatic polysaccharide did not alter the frequency of bone regeneration in the present study, in contrast with results from previous studies showing that other hemostatic agents appeared to increase the frequency of bone repair. Aydinçak et al. [1] reported that bone fracture repair and bone regeneration start from the laminectomy site, whereas Cook et al. [29] found that 50% of laminectomized animals demonstrated bone regeneration by 8 weeks and 75% by 12 weeks. In our study, the rate of bone regeneration following laminectomy with or without hemostatic polysaccharide was 43% at 4 weeks.

Limitations of the present study

There are two main limitations to the present study when attempting to extrapolate to a clinical setting. First, there are likely to be species differences in the inflammatory reaction to surgery as well as to hemostatic polysaccharide. Second, the use of ketorolac postoperatively may have affected the results because of its effects on bleeding and its anti-inflammatory activity [4].

Conclusions

Our results indicate that HaemoCer does not affect the development of EF. Because the application of HaemoCer prevents unwanted bleeding, this hemostatic agent is appropriate for use in rodent spinal surgery. The use of HaemoCer may be considered in spinal surgery with animal evidence to support the lack of significant EF formation.

References

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