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Use of decorin to prevent epidural fibrosis in a post-laminectomy rat model

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ABSTRACT

The formation of epidural fibrosis adjacent to the dura mater is a complex multi-step process that is associated with a marked reduction in tissue cellularity and the excessive deposition of extracellular matrix components. Extensive epidural fibrosis is a major cause of post-laminectomy syndrome. Decorin strongly inhibits fibrosis formation in various tissues via blockade of transforming growth factor-β1. The aim of this study was to investigate the effects of a topical application of decorin on the formation of epidural fibrosis in a rat laminectomy model. Twenty-four female Wistar albino rats (250–350 g) were equally and randomly divided into three groups (control, spongostan and decorin). Laminectomy was performed between the L3 and L5 levels in all rats. The dura mater was directly exposed to spongostan soaked with saline (2 cc/kg) or decorin (100 μg/kg). Four weeks later, the laminectomized spine of the rats was completely removed between the L3 and L5 levels. The extent of the epidural fibrosis and arachnoidal involvement was histopathologically evaluated and graded. Our data revealed that epidural fibrosis was significantly reduced in the group treated with decorin compared to the spongostan and control groups ($P < 0.05$).

Our study demonstrates that the topical application of decorin can be effective in reducing the formation of epidural fibrosis in a simple laminectomy rat model.

1. Introduction

Low-back surgery is used widely throughout the world to treat lumbar herniated-disc symptoms. Common complications of such procedures are epidural fibrosis and post-laminectomy syndrome (Cemil et al., 2009; Guyer et al., 2006). Epidural fibrosis causes compression and/or stretching of the associated nerve root or the dura mater and leads to persistent back and leg pain, known as "post-laminectomy syndrome" or "failed back syndrome," in approximately 24% of patients who undergo lumbar discectomy (Cemil et al., 2009; He et al., 1995; Ismailoglu et al., 2011; Kasimcan et al., 2011). Revision surgery has an increased complication rate, which may include dural tears, nerve root injuries, and epidural bleeding from granulation tissue. These complications result in mostly unsuccessful surgical treatments (Cruccu et al., 2007).

The formation of epidural fibrosis adjacent to the dura mater is a complex multi-step process that is associated with a marked reduction in the tissue cellularity and the excessive deposition of extracellular matrix (ECM) components, including collagen, fibronectin and dermatan sulfate (DS) (Koshiishi et al., 2002; Laurent et al., 2007). Transforming growth factor-beta 1 (TGF-β1) has been found to play a central role in the development of fibrosis following tissue damage by both the initiating fibrosis and facilitating the transdifferentiation of fibroblasts to myofibroblasts (Mohan et al., 2010; Zhu et al., 2007). The proliferation of fibroblasts in the epidural area produces more TGF-β1 and α-smooth muscle actin (α-SMA), a biochemical marker of myofibroblasts, and results in the excessive accumulation of fibrotic tissue (Kozma et al., 2011; Mohan et al., 2010; Sun et al., 2008; Zhu et al., 2007). Decorin is a small, leucine-rich proteoglycan that plays an important role in the cell cycle, tissue development, and remodeling and organization of the ECM (Kalamajski and Oldberg, 2010). The primary function of decorin is modulating the *in vivo* activity of matrix molecules such as collagen, fibronectin, and thrombospondin, in addition to growth factors such as TGF-β (Ferdous et al., 2007; Yamaguchi et al., 1990). Decorin is a natural inhibitor of TGF-β and binds all three isoforms of TGF-β (1-3) with equal efficiency

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(Yamaguchi et al., 1990). Decorin strongly inhibits fibrosis formation in various tissues via blockade of TGF-β1 signaling and improves the healing of injured tissue both histologically and physiologically (Fukushima et al., 2001; Sato et al., 2003).

The prevention of epidural fibrosis has been examined using biological and non-biological materials in animal models. Some of these experimental materials, such as spinal membrane adhesion barrier gel, have been frequently used in routine neurosurgical practice (Ismailoglu et al., 2011; Bora et al., 2001; Jacobs et al., 1980; Kurt et al., 2008). Nonetheless, these materials are frequently associated with a higher cost and require repeated surgery (Lo and Frederickson , 1999). To date, decorin has not been investigated for its potential ability to prevent epidural fibrosis formation in laminectomy models. The purpose of this study was to investigate the effects of topical decorin for the prevention of epidural fibrosis in a rat laminectomy model.

2. Material and methods

2.1. Animals

Adult, female Wistar albino rats weighing 250–350 g were used in this study. All experimental procedures were approved by the ethics committee of Bolu University and minimized the discomfort of the animals during surgery and recovery.

2.2. Surgical procedure and sample preparation

The surgical procedures were performed under general anesthesia induced by intraperitoneal (ip) xylazine (10 mg/kg; Bayer, Istanbul, Turkey) and ketamine hydrochloride (60 mg/kg; Parke-Davis, Istanbul, Turkey). After the lower back of each rat was shaved, the surgical site was sterilized with povidone. All of the surgical procedures were performed by the first author. A longitudinal midline skin incision was performed over the L3–L5 levels using a surgical microscope with a magnification of 16× (Zeiss OPMI 1, Carl Zeiss Meditec, Oberkochen, Germany). The lumbosacral fascia was incised, and the paraspinous muscles were dissected subperiostally to expose the L3–L5 laminae. A total L3–L5 laminectomy and flavectomy were performed, and the epidural fat tissue was removed, leaving the dura mater clean and fully exposed. Hemostasis was achieved using cotton pads. The rats were then randomly allocated into 3 groups with 8 rats per group. After treatment, the wounds were closed in anatomical layers using the same suture material (Prolen polypropylene sutures, Ethicon, Ethicon Endo-Surgery, Inc., Cincinnati, OH, USA) in each animal. The animals were euthanized on the 30th post-operative day using a lethal dose of pentobarbital (60 mg/kg; IE Ulagay, Istanbul, Turkey). There were no complications, wound infections, or any adverse effects related to using decorin. The vertebral columns of the rats were removed en-bloc, including the whole laminectomy area between the L3 and L5 levels. The specimen was then placed into 10% buffered formalin.

2.3. Experimental groups

Control group ($n=8$): only a laminectomy was performed; no treatment was given.

Decorin group ($n=8$): 100 μg/kg decorin (Sigma-Aldrich, St Louis, MO, USA) was applied with a spongostan (Ethicon, Ethicon Endo-Surgery, Inc. Cincinnati, OH, USA) soaked with 0.5 ml of the solution and was left on the dura mater. This dosage of decorin was selected based on earlier studies (Alan et al., 2011; Zhu et al., 2007).

Spongostan group ($n=8$): a spongostan was soaked with 2 cc/kg of saline solution and was left on the dura mater.

2.4. Evaluation of epidural fibrosis

The specimens were decalcified by ethylenediamine tetra-acetic acid (EDTA) (R&D Systems Inc., Minneapolis, MN, USA). After complete decalcification, the specimens were dehydrated and embedded in paraffin. The thickness of the dura mater, arachnoidal involvement and epidural fibrosis was measured at 3 points of the en-bloc laminectomized spines (L3, L4, and L5) of the rats, as described by Cemil et al. (2009). The first sample with the thick of 5 mm was harvested from the midpoint of the laminectomy defect. The second sample with the thick of 5 mm was obtained 1 cm to the right of the first sample, and the third sample with the thick of 5 mm was obtained 1 cm to the left of the first sample. Sections of 10 μm were obtained in the axial plane and stained with Masson trichrome. The sections were examined using a Nikon eclipse 80i microscope and photographed using a Nikon DS-Fi1 camera. All laminectomized spine sections were evaluated in a blind manner by a single pathologist who analyzed the thickness of the dura mater, density of fibrosis, and arachnoidal involvement. Quantitative morphometric analysis was performed on sections using the Nikon Nis elements D 3.1 Digital Analyzing System. Measurements were conducted at a magnification of 100×.

Mean values were used for statistical analysis. Epidural fibrosis was graded based on the scheme devised by He et al. (1995): *Grade* 0: dura mater was free of scar tissue; *Grade* 1*:* only thin fibrous bands were observed between the scar tissue and dura mater; *Grade* 2*:* continuous adherence was observed in < two-thirds of the laminectomy defect; and *Grade 3:* scar tissue adherence was large, affecting > two-thirds of the laminectomy defect, or the adherence extended to the nerve roots. The presence of arachnoidal involvement was also noted.

2.5. Statistical analysis

Data analysis was performed using SPSS for Windows version 11.5 (SPSS Inc., Chicago, IL, USA). Descriptive statistics for ordinal variables were represented as the 25th–75th percentiles, and categorical variables were represented as the percentage (%) of rats. The Kruskal–Wallis test was used for the determination of statistical significance between groups for the density of the epidural fibrosis. If the Kruskal–Wallis test was statistically significant, the Conover non-parametric multiple comparison test was used to determine specific group differences. The presence of arachnoidal involvement was statistically analyzed using a likelihood ratio test. Statistically significant *p*-values were defined as $P<0.05$.

3. Results

3.1. Wound healing and complication rates related to the procedure

There was no mortality and morbidity related to the procedure. Decorin did not affect the surrounding tissue or wound healing in any rats. No wound infection, erythema, hematoma or CSF leakages were observed. All of the animals were ambulatory at the time of euthanasia.

3.2. Assessment of the thickness of the dura mater, epidural fibrosis and arachnoidal involvement

The mean thickness of the dura mater was measured as 9.28 ± 3.39 μm in the spongostan group, 8.69 ± 2.32 μm in the decorin group and 14.70 ± 4.14 μm in the control group. The differences in the thickness of the dura mater between the treatment groups and the