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Original Investigation

Prevention of Epidural Fibrosis Using Ranibizumab in a Postlaminectomy Rat Model

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ABSTRACT

AIM: One of the most significant reasons for persistent low back pain experienced after spinal surgery is epidural fibrosis seen after laminectomy procedures. This study shows the effects of Ranibizumab on spinal epidural fibrosis in the laminectomy area by blocking the effect of vascular endothelial growth factor.

MATERIAL and METHODS: Twenty Wistar rats were used in this study. Rats were divided into two groups; a control group and a ranibizumab group. Only laminectomy was performed to the control group. In the ranibizumab group, 0.6 mg/kg ranibizumab diluted in 0.9% NaCl with the ratio of 1:10 was applied topically. Three weeks later, the vertebral columns were resected en bloc including the whole laminectomy area in both groups and evaluated histopathologically. Results were compared using statistical tools.

RESULTS: Based on the statistical analysis, our data show that less epidural fibrosis was seen in the ranibizumab group compared to the control group ($P<0.05$).

CONCLUSION: Topically applied Ranibizumab is significantly effective in preventing epidural fibrosis in rats occurred after laminectomy.

KEYWORDS: Epidural fibrosis, Laminectomy, Ranibizumab

INTRODUCTION

It is currently known that one of the most significant reasons for persistent low back pain experienced after spinal surgery is epidural fibrosis seen after laminectomy procedures (2,17,18,28,34,35,37). Epidural fibrosis is a natural process of laminectomy performed during surgery (17).

According to studies in the United States, laminectomy and laminotomy are performed on approximately 250000 people per year because of lumbar disc herniation. 30000-40000 of these result in failed back surgery (6,12,13). If we consider the additional laminotomies and laminectomies performed for many reasons such as spinal trauma, spinal stenosis, scoliosis, tumor etc., we can appreciate that many patients face the risk of epidural fibrosis. The rate of persistent low back pain due to epidural fibrosis seen post laminotomy and laminectomy is 1-40%. Previous studies have shown that fibrotic tissue causing pressure on surrounding anatomic structures lead to clinical sequelae (17,18,27).

Many methods and agents have been used to prevent the development of epidural fibrosis so far; however, none of them has been widely accepted in routine clinical practice (26).

Ranibizumab, which has the effect of Anti-Vascular Endothelial Growth Factor (VEGF) that is used in macular degeneration of the eye, has been shown to decrease new vascular formation in the scar tissue by inhibiting the effect of VEGF (44).

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Table I: Scoring of the Scar Amount (20)

Grade 0	No scar tissue adhered to dura.
Grade I	Only a thin fibrous band between the scar tissue and dura.
Grade II	Ongoing adhesion is observed but it composes less than 2/3 of the laminectomy defect
Grade III	Scar tissue adhesion is larger or composing more than 2/3 of the laminectomy defect and/or extending to the nerve root

Table II: Histological Results of Epidural Fibrosis Grades

Epidural Fibrosis Grade	Control Group	Ranibizumab Group
Grade 0	0	1
Grade I	0	4
Grade II	4	3
Grade III	6	2

In this study, we analyzed whether ranibizumab had an inhibitory effect on the development of epidural fibrosis, which develops as a natural result of laminectomy, in the epidural fibrosis model developed in post laminectomy rats.

MATERIAL and METHODS

This study was conducted at the Experimental Animals Research Laboratory of the Ankara Teaching and Research Hospital, upon the approval of the Animal Experimentation Local Board of Ethics of Ankara Teaching and Research Hospital dated 08/10/2013-227.

In our study, 20 male Wistar rats aged between 8-12 months with an average weight of 200-250 gr were used. The rats were categorized into 2 groups. A single dose of 50 mg/kg of ceftriaxone (Rocephin, Roche, Turkey) was given intraperitoneally to the rats 30 minutes before surgery for prophylactic reasons. Anesthesia was induced by intramuscular ketamine hydrochloride (25 mg/kg; Ketalar, Pfizer, Istanbul, Turkey) and Xylazine (5 mg/kg; Rompun, Bayer, Istanbul, Turkey). The rats were fixed to the operating table in prone position. After the fixation, the operation site was brushed with povidone iodine scrub (Medicabrush; 4% chlorhexidine soap, Medica BV, Netherlands) for 10 minutes and disinfected with povidone-iodine (Poviod; 10% polyvinylpyrrolidone-iodine complex, Saba, Turkey) solution. The surgical site was covered with sterile covers. By means of a median skin incision the L1-S1 vertebrae were spaced. Paravertebral muscles were stripped by microdissection. Total laminectomy was performed on L3, L4, L5 vertebrae. Ligamantum flavum and epidural fat tissue were cleaned. Lumbar epidural space was exposed by bipolar after hemostasis. No dural tear and injury in the nerve root was observed during the procedure in any of the rats. The 1st group of the rats had laminectomy only. The 2nd group received 0.6 mg/kg of ranibizumab (Lucentis®, Roche), which was diluted by 1/10 with 0.9% of NaCl and applied topically on the epidural space with cotton for 5 minutes. The soaked cotton was removed from the surgical site after 5 minutes and the layers were closed in the anatomical plane. All the surgical procedures were performed with 16 times magnification by the OpMi (Carl Zeiss, Germany) microscope. None of the subjects had infection. The rats were sacrificed after 3 weeks with intraperitoneal high dose of 75-100 mg/kg sodium thiopental (Pentothalsodium, Abbott, Italy). The vertebral column was removed including the laminectomy site. The material was fixed with 10% of formal (4% formaldehyde). They were decalcified with 30% formic acid for 2 days. Serial sections were collected from each block for histopathological analysis. They were stained with hematoxylin eosin (HE). The preparations were examined under light microscope. Photos of the scar tissue were taken using the ZeissImager M2" microscope. Staging of the epidural scar tissue was done according to plan described by He et al. (20) (Table I).

Statistical Analysis

Data analysis was performed by using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL, United States). The differences between control and Ranibizumab groups regarding epidural fibrosis grade were evaluated by the Mann-Whitney U test. A p value less than 0.05 was considered statistically significant.

RESULTS

In our study, no dura nerve injury or infection was defined in any of the subjects. Epidural fibrosis development was found to be significantly lower in ranibizumab group compared to the control group (p=0.016) (Figure 1). Grade II epidural fibrosis was defined in 4 rats and grade III was defined in 6 rats in the control group (Figure 2). In the ranibizumab group, grade 0 epidural fibrosis was defined in 1 rat (Figure 3), grade I in 4 rats, grade II in 3 rats and 2 rats had grade III epidural fibrosis (Table II).

DISCUSSION

Epidural fibrosis is seen as a natural consequence of laminotomy and laminectomy performed due to many reasons (3,20). Failed Back Surgery Syndrome (FBSS), in other words post laminectomy syndrome, composes a subgroup of chronic low back pain. It covers the group of patients with persistent low back and leg pain after spinal laminectomy and laminotomy (16). One of the most important reasons of FBSS is the development of epidural fibrosis after laminectomy and laminotomy (4,19,23,38,41).

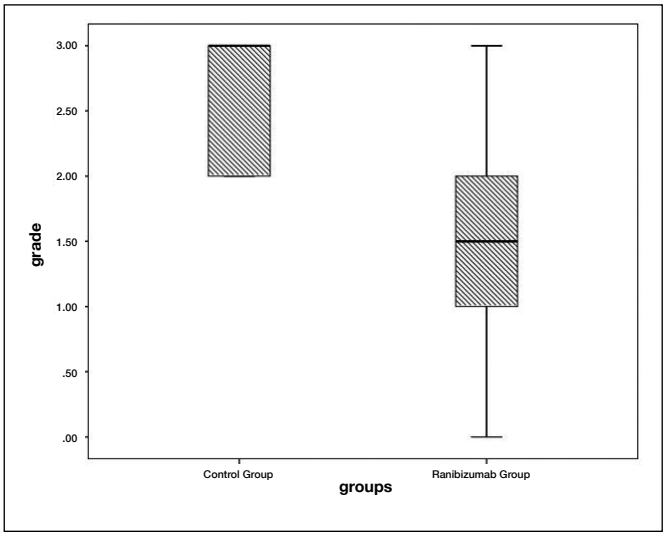

Figure 1: Epidural fibrosis grades shown with box plot. The ranibizumab group had a significantly lower epidural fibrosis grade (p=0.03).

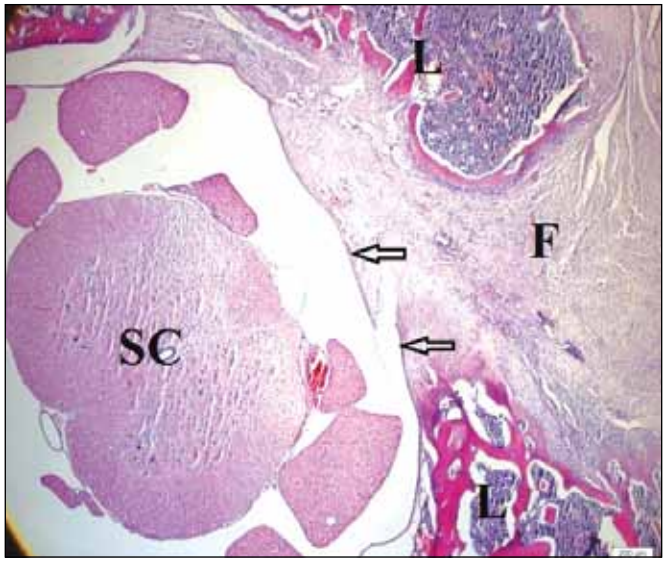

Figure 2: Microscopic image shows Grade III fibrosis as observed in the control group. The epidural fibrosis adhered to the underlying dura mater. **L=** lamina; **F=** fibrosis; **SC=** spinal cord; **Black arrows=** dura mater. Scale bar=200 µm.

Epidural fibrosis develops with the combination of fibroblasts and elements including inflammatory cells and collagen produced by fibroblasts. As a result of adhesions stemming from epidural fibrosis formation, a pressure and tension effect is seen on the spinal cord and nerves (4,19,38). This adhesion may disrupt the arterial supply, venous drainage and axoplasmic transport in the nerve fibers. Although there is no consensus about the mechanism of the scar tissue in the etiology of the pain, it is reported that the adhesions cause pressure on the surrounding anatomical structures and increase the sensitivity of the nerve tissue in addition to the limitation of mobility in the nerve root (10,18,30,45).

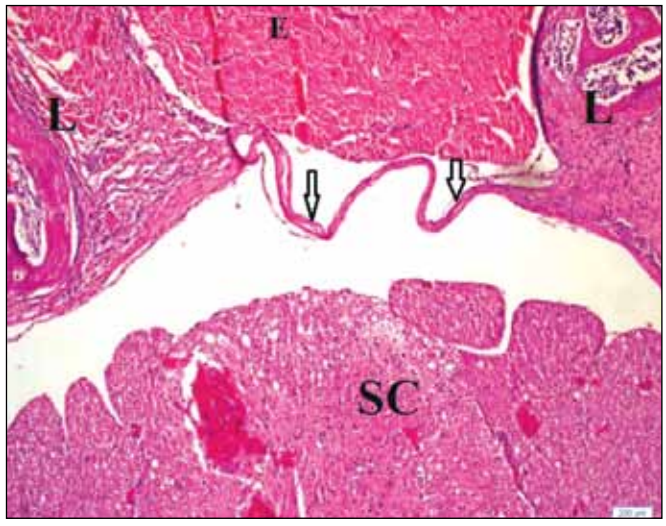

Figure 3: Microscopic image shows Grade 0 fibrosis as observed in the ranibizumab group. The dura mater was free of scar tissue **E:** epidural area **(**No scar tissue adhered to dura**), SC=** spinal cord; **L=** lamina; **Black arrows=** dura mater. Scale bar= 200 µm.

Today, the pathophysiological process of epidural fibrosis formation is still under debate. Many factors are set forth in clinical and animal studies. Such factors as individual variations in scar tissue and its formation, postoperative hematoma, laminectomy technique and amount of bone removed in laminectomy are blamed (34). It is shown that fibroblast migration into the surgical field plays a key role in the formation of epidural fibrosis (26). Fibroblasts originate from paravertebral muscles and are transported to the surgical site with blood causing strong adhesions in the tissue (26).

There is an increase in vascular permeability during wound healing and in the early period of repair (9). This event allows the storage of fibrin rich extracellular matrix proteins for cell migration and proliferation (34).

Together with the post-spinal surgery epidural fibrosis, low back pain is experienced due to sciatic irritation (22). Many studies have been conducted so far in order to decrease epidural fibrosis. Numerous methods such as Silastic-Dacron gelatin sponge, animal collagen membranes, Adcon-L, autologous lipid graft, local cortisone application, tenoxicam application, omental graft, and bevacizumab 5-Fluorouracil combination have been tried. However, they are not totally used in routine practice (8,11,14,25,29,36).

VEGF is a strong angiogenic cytokine that includes several sub-groups named VEGF-A, VEGF-B, VEGF-C, VEGF-D and VEGF-E. It has been proven that VEGF protects the endothelial cells from radiation and stress-induced apoptosis, ensures the survival of cancer cells (43) and is actively effective in cell regeneration, fibroblast function, wound healing, and reaction to inflammation (21). VEGF plays a role in the formation of adhesions and helps the vascularization process in the postoperative damaged area (24).

Today, it is known that VEGF production is stimulated by some environmental factors, growth factors, oncogenes, cytokines and hormones. The VEGF that is produced is bound to the endothelial cell surface and intracellular tyrosine kinase is activated. VEGF-A is the form that is primarily responsible for angiogenesis and vascular permeability. VEGF-A has 9 isoforms according to the number of amino acids (1,33). Anti-VEGF treatment regimens are based on this basic information. The studies show that the anti-VEGF agents prevent tissue adhesions that are formed secondary to these events by decreasing endothelial cell angiogenesis and vascular permeability (24).

Ranibizumab (Lucentis®, Novartis) is a molecule containing the antigen-binding fragment of the anti-VEGF antibody that is produced by recombinant DNA technology (44). It binds to all of the isoforms of VEGF-A and eliminates their effect. Ranibizumab was approved by the FDA for the treatment of choroidal neovascularization in wet age-related macular degeneration (AMD) intravitreally (7). The clinical efficacy and reliability of ranibizumab have been shown in various studies (40). The fact that anti-VEGF agents decrease adhesions by decreasing new vessel formation in damaged tissue by an anti-VEGF feature is well documented in animal studies of the eye, abdominal surgery, and laminotomy models (5,31). Hypertension and renal and cardiac toxicity have been defined in cases as a consequence of the administration of anti-VEGF agents for various reasons. The mechanism is not entirely known (15).

Before the approval of ranibizumab by the FDA in 2006, premise drug bevacizumab, an anti-VEGF agent, was approved by the FDA in 2004 for the treatment of metastatic cancer of the colon or rectum as an intravenous infusion (15). Rosenfeld pioneered the off-label use of bevacizumab in the eye after previous data suggested its efficacy in treatment of wet AMD intravenously (32,39). Bevacizumab is a larger molecule with a longer half-life than ranibizumab. Both ranibizumab and bevacizumab inhibit all biologically active forms of VEGF (46). Although both drugs have been shown independently to be effective in macular degeneration as anti-VEGF agents, Subramanian et al. showed in a prospective, randomized, double-masked study that the central macular thickness measured by optical coherence tomography (OCT) was greater in the ranibizumab group compared with the bevacizumab group. There was also a great improvement in central foveal thickness in the ranibizumab group. However, they found no difference in visual and anatomic outcomes at 1 year (42). Today, ranibizumab is the current gold standard as an anti-VEGF in the treatment of AMD in the United States (42).

In addition, in a study conducted with bevacizumab on rat models, epidural fibrosis was reported to decrease. Histopathologic examinations showed that epidural fibrosis was significantly less in the group where bevacizumab was used (24). Further studies can be done to clarify the differences between ranibizumab and bevacizumab on epidural fibrosis.

CONCLUSION

Our study shows the effects of ranibizumab, an anti-VEGF agent, in preventing epidural fibrosis in post laminectomy rat models. According to the histopathological analysis, we observed that the ranibizumab-administered group had significantly less epidural fibrosis compared to the control group. This agent, which is used on a routine basis for other indications, is investigated for the first time for its efficacy in epidural fibrosis. We believe that further laboratory and clinical studies should be conducted to support our study.

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