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The Effect of Bevacizumab on Spinal Epidural Fibrosis in a Postlaminectomy Rat Model

Postlaminektomi Rat Modelinde Bevacizumabın Spinal Epidural Fibrozise Etkisi

Mete KARATAY[1], Yavuz ERDEM[1], Ender KOKTEKIR[2], Yavuz Selim ERKOC[1], Muzaffer CAYDERE[3], Mehmet Akif BAYAR[1]

[1]Ministry of Health, Ankara Training and Research Hospital, Department of Neurosurgery, Ankara, Turkey
[2]Selcuk University, Faculty of Medicine, Department of Neurosurgery, Konya, Turkey
[3]Ministry of Health, Ankara Training and Research Hospital, Department of Pathology, Ankara, Turkey

Correspondence address: Mete KARATAY / E-mail: lexel26@hotmail.com

ABSTRACT

AIM: Spinal epidural fibrosis is an inherent result of surgical trauma after laminectomy. The conditions in which epidural fibrosis is excessive are in the etiology of failed back syndrome. There have been many attempts to prevent formation of epidural fibrosis. Bevacizumab which is an anti-angiogenic medication, inhibits the effect of VEGF and thereby decreases the new blood vessel formation and as a result prevents adhesions. This study shows the effect of bevacizumab on spinal epidural fibrosis developing after laminectomy in rats.

MATERIAL and METHODS: In this study, 20 Wistar rats were used. Rats were divided into two groups; a control group, and a bevacizumab group. Three-level laminectomy was performed on the rats. Rats in the control group only had the laminectomy. In the bevacizumab group, 2.5 mg/kg bevacizumab diluted in 0.9% NaCl with a factor of 1:10 impregnated on cotton was applied on the dura topically for 5 minutes. Three weeks later, rats were sacrificed for histopathologic examination. Epidural fibrosis tissue was graded following sacrifice.

RESULTS: Statistically, it was found that the bevacizumab group had significantly less epidural fibrosis compared to the control group ($p<0.05$).

CONCLUSION: Bevacizumab reduced the spinal epidural fibrosis significantly that developed in rats after laminectomy via its anti-VEGF effect by blocking VEGF receptors.

KEYWORDS: Spinal epidural fibrosis, Failed back syndrome, Laminectomy, Bevacizumab, VEGF (vascular endothelial growth factor)

ÖZ

AMAÇ: Spinal epidural fibrozis laminektomi sonrası gelişen cerrahi travmanın doğal bir sonucudur. Epidural fibrozisin fazla olduğu durumlar failed back sendromu nedenleri arasında yer almaktadır. Epidural fibrozisin engellenmesi amacıyla bir çok materyal denenmiştir. Antianjiogenetik bir ilaç olan Bevacizumab VEGF 'ün etkisini engelleyerek hasarlı dokuda yeni damar oluşumunu ve dolayısıyla yapışıklık oluşmasını engellemektedir. Bu çalışma, Bevacizumab'ın ratlarda laminektomi sonrası gelişen spinal epidural fibrozis üzerine olan etkisini göstermiştir.

YÖNTEM ve GEREÇLER: Bu çalışmada, 20 adet Wistar cinsi rat kullanıldı. Kontrol ve Bevacizumab olmak üzere ratlar 2 ayrı gruba ayrıldı. Ratlara 3 seviye laminektomi yapıldı. Kontrol grubuna sadece laminektomi yapıldı. Bevacizumab kullanılan gruba ise pamuğa emdirilmiş 2,5 mg/kg bevacizumab %0,9 NaCl ile 1:10 sulandırılarak cerrahi alanda dura üzerine topikal olarak 5 dakika süre ile uygulandı. 3 hafta sonra ratlar sakrifiye edilerek histopatolojik incelendi. Epidural fibrozis dokusu derecelendirildi.

BULGULAR: Kontrol grubu ile karşılaştırıldığında Bevacizumab kullanılan grupta epidural fibrozisin istatiksel olarak anlamlı derecede azaldığı görüldü ($p<0,05$).

SONUÇ: Bevacizumab VEGF reseptörlerini bloke edip anti-VEGF etkisi ile laminektomi yapılmış ratlarda gelişen spinal epidural fibrozisi anlamlı derecede azaltmıştır.

ANAHTAR SÖZCÜKLER: Spinal epidural fibrozis, Failed back sendromu, Laminektomi, Bevacizumab, VEGF(vaskuler endothelial growth factor)

INTRODUCTION

Presently, one of the most common surgeries in neurosurgical practice is laminectomy or discectomy for lumbar disc herniation. Failed back syndrome is defined as a situation when back and/or leg pain complaints won't alleviate or last incrementally. In a considerable amount of cases with failed back syndrome, it is difficult to figure out the reason for the pain after patients are diagnosed with the etiology of pain. When there is a diagnosis, the most common reasons are; spinal stenosis, recurrent disc herniation, spinal instability, root degeneration, pseudoarthrosis, foreign body reaction, operation on the inaccurate spinal level, and epidural fibrosis (1). Epidural fibrosis developing after surgery is actually an inherent result of surgical trauma. However, the

degree of epidural fibrosis can be much more severe in some cases in the postoperative period. In cases where epidural fibrosis develops, radicular pressure or stretching will give rise to continuation of the pain or increases in the pain (7). Localization and size of this developing fibrosis can be shown by MRI (14). There have been many substances or materials utilized in attempt to prevent or keep epidural fibrosis that develop after lumbar disc herniation to a minimum (5, 11). However, the outcomes of these studies are not satisfactory.

Angiogenesis can be defined as the development of new vessels during the wound healing process. In accordance with this definition, one can think that the degree of fibrotic tissue will be directly proportional to the degree of angiogenesis. VEGF (vascular endothelial growth factor) has been shown to be a very special factor in the formation of angiogenesis apart from other factors (20, 24).

VEGF is a potent angiogenic cytokine that affects the formation of fibrosis. VEGF and VEGF receptors play a major role in the formation of new vessels. This happens by the development of new vessels that feed the areas of the tissue distraction which aids in wound healing. Anti-VEGF is responsible for slowing wound healing by decreasing fibroblast migration and proliferation (12). Bevacizumab blocks VEGF receptors in damaged tissue that renders the VEGF inactive which will result in decreasing new vessel formation by decreasing angiogenesis so that fibrotic tissue formation is hindered (13, 18).

In this study, the effect of bevacizumab as an angiogenesis inhibitor substance on the formation of spinal epidural fibrosis formation in rats was investigated.

MATERIAL and METHODS

This study was performed by the approval of local ethical institution for animal experiments in Ankara Training and Research Hospital as of 24/02/2011.

In this study, a total of 20 Wistar rats were used. The average weight of the rats was 200-250 grams, with an average age of 8-12 months. All rats were male. Rats were divided into two groups, 10 rats in each group. Each rat was numbered individually. Anesthesia was induced by ketamine hydrochloride (25 mg/kg; Ketalar, Pfizer, Istanbul, Turkey) and Xylazine (5 mg/kg; Rompun, Bayer, Istanbul, Turkey) intramuscularly. Rats were placed in the prone position and their backs were shaved. The surgical field was sterilized by povidone-iodine (Batticon, Adeka Pharmaceuticals Istanbul, Turkey). A median skin incision was performed from the L1 to the S1 vertebrae. The paraspinal muscles were dissected as two-sided by microdissection. Total laminectomy was performed on L3, L4, and L5 vertebrae. Ligamentum flavum and epidural fat tissue were cleaned. A bipolar coagulator was used for homeostasis. Close attention was paid not to traumatize the dura and the nerve roots. No dura or nerve root trauma occurred in any of the rats. Subjects were divided into two groups. The control group only had the laminectomy. In the bevacizumab group, 2.5 mg/kg bevacizumab (Avastin 25 mg/mL, Roche, Basel, Switzerland) diluted with 0.9% sodium chloride with a factor of 1:10 impregnated in cotton was applied on the dura topically for 5 minutes (3). Five minutes later, the cotton was removed from the surgical field, anatomical levels were closed and surgery was ended.

All surgical procedures were performed by an OpMi (Carl Zeiss, Germany) made microscope and with a magnification of X16. Subjects were kept alive for 3 weeks and then sacrificed by applying 75-100 mg/kg thiopental sodium (Pentothal sodium, Abbott, Italy). No infection in the surgical field was detected in the subjects. Vertebral columns were resected en bloc including the whole laminectomy area (L3, L4, L5 vertebrae). Materials were fixed in 10% formal (4% formaldehyde) and then decalcified for 2 days in 30% formic acid at which time tissue processing was done. Four-micron-thick sections that were obtained from previously prepared paraffin blocks were stained by hematoxylin and eosin (H&E). These preparations were examined under light microscope. Fibrous tissue was examined and photographed under "Zeiss Imager M2" microscope. Grading of epidural fibrosis tissue was performed according to the definition of He et al. (11) (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 bevazicumab groups regarding for epidural fibrosis grade was evaluated by Mann-Whitney U test. A p value less than 0.05 was considered statistically significant.

RESULTS

No infections were detected in the surgical field in the subjects. There was no dura or nerve root trauma in any of the subjects during surgery. As a result of histopathologic examinations, epidural fibrosis was significantly less in the group that used bevacizumab compared to the control group ($p<0.05$) (Figure 1).

Table I: Grading of Epidural Fibrosis Tissue

Grade 0	The dura mater was free of scar tissue
Grade I	Only thin fibrous bands between scar tissue and dura mater were observed
Grade II	Continuous adherence was observed but was less than two thirds of the laminectomy defect
Grade III	Scar tissue adherence was large, more than two thirds of the laminectomy defect, and/or extended to the nerve roots

In the control group, 3 rats had grade II and 7 rats had grade III epidural fibrosis (Figure 2). In the group that used bevacizumab however, 5 rats had grade I (Figure 3), 4 rats had grade II, and 1 rat had grade III epidural fibrosis. In the control group, no rats had grade 0 or grade I epidural fibrosis. In the group that used bevacizumab, no subjects had grade 0 epidural fibrosis (Table II).

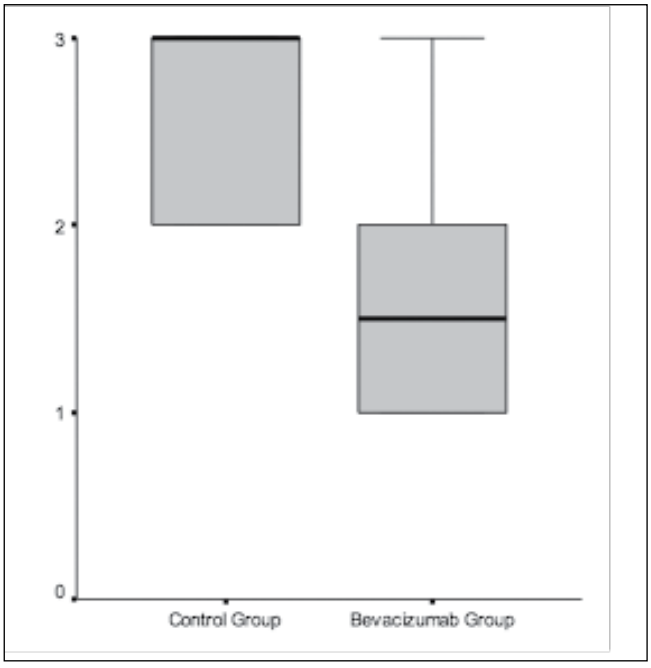

Figure 1: Box-plot for epidural fibrosis grades. The bevacizumab group had a significantly lower epidural fibrosis grade (p=0.003).

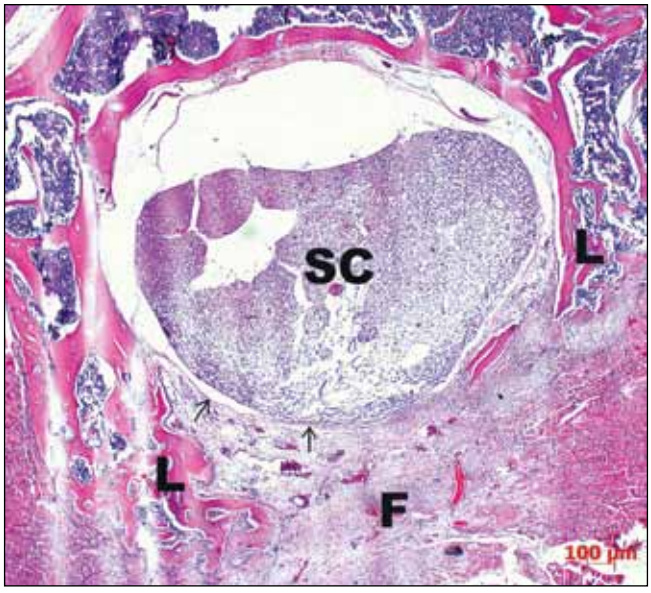

Figure 2: Photomicrograph showing Grade III fibrosis as observed in the control group. The epidural fibrosis was adhered to the underlying dura mater and spinal cord. **L=** lamina; **F=** fibrosis; **SC=** spinal cord; **Black arrows=** dura mater. Scale bar=100 µm.

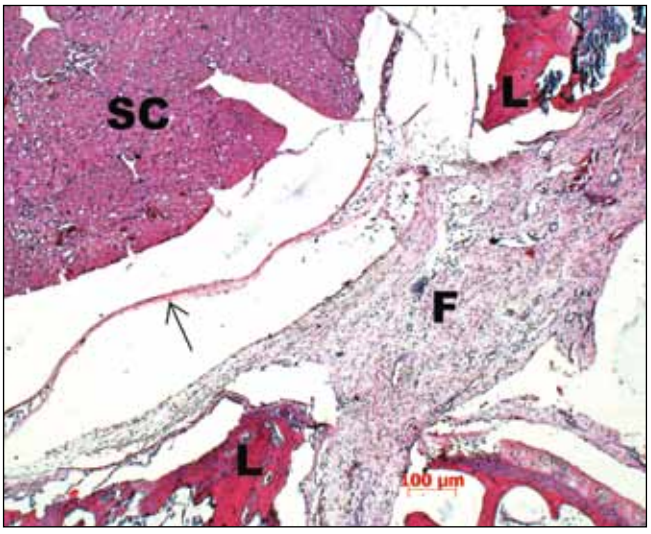

Figure 3: Photomicrograph showing Grade 1 fibrosis as observed in the bevazicumab group. No direct contact between the underlying spinal cord and the epidural fibrosis tissue is evident. **F=** fibrosis; **SC=** spinal cord; **L=** lamina; **Black arrow=** dura mater. Scale bar=100 µm.

Table II: Histological Results of Epidural Fibrosis Grades

Epidural Fibrosis Grade	Control Group	Bevacizumab Group
Grade 0	0	0
Grade I	0	5
Grade II	3	4
Grade III	7	1

***p*=0.003; Mann-Whitney U test.**

DISCUSSION

Failed back syndrome denotes a clinical picture resulting from unfavorable results of lumbar disc surgery. One of the reasons for failed back syndrome is the epidural fibrosis that happens in the operation site. Epidural fibrosis which is an inherent result of the surgery can press on the nerve roots causing stretching that will result in the continuation or increase of the pain (15).

Epidural fibrosis develops as a result of invasion of the postoperative hematoma by the dense fibrotic tissue which develops on the level of the fibrous periosteum and the deep paravertebral muscles. This fibrosis can extend to the nodal canal and can abut the nerve root and dura mater. Mechanical radicular pain develops due to the fibrosis in the nerve roots and dorsal root ganglia. There is generally no problem in the early postoperative period. In the following weeks or months, pain can arise even with sensory or motor deficits. Complaints generally start between 3 to 6 months and increase gradually and incrementally (2, 25).

This physiologic scar tissue is exacerbated by technical errors during the operation, keloid reaction, and hematomas.

As a result of this, a hypertrophic surrounding membrane develops. That membrane has been defined or named as the postlaminectomy membrane by LaRocca and Macnab (16). This membrane develops as a result of the progression of erector muscles into the spinal canal and the dissection of the intraspinal hematoma and epidural fat tissue. This membrane is considered to be the cause for lumbar pain and sciatic irritation that adversely affect prognosis of some patients after lumbar vertebrae surgery (23). Until today, many materials have been tried to prevent or keep at a minimum this epidural fibrosis that develops after lumbar disc herniation surgery. For this purpose, many experimental and clinical studies have been performed. The most frequently used materials include Silastic-Dacron gelatin sponge, animal collagen membranes, Adcon-L, autologous lipid graft, omentum graft, and locally applied cortisone. However, the outcomes of these applications are not so appealing (4, 5, 17, 19, 21, 22).

VEGF (vascular endothelial growth factor) is a strong angiogenic cytokine. At the same time, VEGF protects the endothelial cells from radiation or stress-induced apoptosis. VEGF helps cancer cells survive and resist therapy (24). VEGF also takes direct and active participation in tissue regeneration including remodeling, fibroblast function, wound healing, and inflammatory reactions (12). VEGF plays role in adhesion formation and help regenerate new vessels that provide circulation to the damaged areas from surgery (8).

Bevacizumab is a comprehensive murine derived monoclonal antibody against VEGF molecule (93% of amino acid sequence is from human and 7% from murine) that is applied to humans (38). It binds to all biologically active VEGF isoforms. It also inhibits binding of this cytokine to its receptors (VEGFR-1 and 2 ligands) (13). In rat models, it was shown that anti-VEGF antibodies inhibit human cancer xenografts and decreases the number and extent of the metastasis (10). Bevacizumab, by inhibiting VEGF action, renders cancer cells susceptible to cytotoxicity of the chemotherapy and the hypoxia derived from therapy (6). Bevacizumab neutralizes all the biologic effects of VEGF including, endothelial cell angiogenesis, improvement of vascular permeability and the increase in angiogenesis (15). Bevacizumab is currently used for colorectal cancer patients systematically. Side effects are relatively minor (mild-to-moderate degree of hypertension and increase in thrombosis induced patient population) (9). The fact that bevacizumab decreases adhesions by decreasing new vessel formation in damaged tissue by an anti-VEGF feature is well documented in animal studies of eye and abdominal surgery (3, 18).

In this study, we investigated the anti-adhesion effect of bevacizumab which is an antiangiogenic medication on epidural fibrosis that develops after laminectomy in rats. In the wake of histopathologic examinations, the bevacizumab group had significantly less epidural fibrosis compared to the control group ($p<0.05$) (Figure 1). In the control group, 3 rats had grade II, and 7 rats had grade III (Figure 2) epidural fibrosis. In the bevacizumab group, 5 rats had grade I (Figure 3), 4 rats had grade II and 1 rat had grade III epidural fibrosis. In the control group, none of the rats had grade 0 or grade I epidural fibrosis. In the bevacizumab group, none of the subjects had grade 0 epidural fibrosis (Table II).

This study has shown that bevacizumab decreases epidural fibrosis significantly. This finding should be supported by other experimental studies for its clinical application.

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