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

Postlaminektomi Rat Modelinde Etanerseptin Spinal Epidural Fibrozis Üzerine Etkisi

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ABSTRACT

AIM: The formation of epidural fibrosis adjacent to the dura mater after posterior spinal surgery is a normal reaction of the body to surgery. Extensive epidural fibrosis is one of the important causes of postlaminectomy syndrome. Etanercept inhibits tumor necrosis factor-alpha and decreases fibroblast migration. Thus, etanercept prevents the formation of fibrosis. The aim of this study was to investigate the effects of topical application of etanercept on epidural fibrosis after laminectomy in a rat model.

MATERIAL and METHODS: Twenty-four Wistar rats were equally and randomly divided into three groups (control, spongostan and etanercept). Laminectomy was performed between L3 and L5 in all the rats. Spongostan soaked with saline (0.1 mg/kg) and etanercept (300 µg/kg) was directly exposed to and left on the dura mater. Four weeks later, the vertebral columns of the rats were removed en bloc between T10 and L5, and epidural fibrosis and arachnoidal involvement were evaluated and graded histopathologically.

RESULTS: Our data revealed that epidural fibrosis was reduced significantly in the rats treated with etanercept, compared to the control groups ($p<0.05$).

CONCLUSION: Our study demonstrated that topical application of etanercept can be effective in reducing epidural fibrosis in rats after laminectomy.

KEYWORDS: Epidural fibrosis, Etanercept, Failed back surgery, Fibroblast, Laminectomy, Post-laminectomy syndrome, Rat

ÖZ

AMAÇ: Posterior spinal cerrahi sonrası duramatere yapışık epidural fibrozis formasyonu vücudun cerrahiye karşı vermiş olduğu normal bir reaksiyondur. Aşırı epidural fibrozis postlaminektomi sendromuna yol açan önemli nedenlerden biridir. Etanersept tümör nekrozis faktör alfay'ı inhibe ederek fibroblast migrasyonunu engeller ve sonuç olarak fibrozis gelişmesini önler. Bu çalışmanın amacı; topikal uygulanan etanersept'in rat laminektomi modelinde gelişen epidural fibrozis üzerine etkisini araştırmaktır.

YÖNTEM ve GEREÇLER: 24 Wistar rat rastgele ve eşit olarak üç gruba (Kontrol, spongostan ve etanersept) ayrıldı. Bütün ratlara L3-L5 laminektomi yapıldı. Spongostana emdirilmiş salin (0.1mg/kg) ve etanersept (300 µg/kg) direkt duramatere uygulandı ve duramater üzerinde bırakıldı. 4 hafta sonra ratların T10-L5 arası omurgaları enblok olarak çıkartılarak, epidural fibrozis ve araknoidal tutulum histopatolojik olarak incelendi ve derecelendirildi.

BULGULAR: Kontrol grubu ile karşılaştırıldığında topikal uygulanan etanersept grubunda epidural fibrozisin istatistiksel olarak anlamlı derecede azaldığı görüldü ($p<0.05$).

SONUÇ: Çalışmamız; topikal uygulanan etanerseptin laminektomi yapılmış ratlarda gelişen epidural fibrozisin azaltılmasında etkili olduğunu göstermiştir.

ANAHTAR SÖZCÜKLER: Başarısız bel cerrahisi, Epidural fibrozis, Etanersept, Fibroblast, Laminektomi, Poslaminektomi sendromu, Sıçan

INTRODUCTION

The formation of epidural fibrosis adjacent to the dura mater after posterior spinal surgery is a normal reaction of the body to surgery (7, 8). Epidural fibrosis causes compression and/or stretching of the associated nerve root or the dura mater and can lead to persistent back and leg pain, known as "post-laminectomy syndrome" or "failed back syndrome," in 24% of patients (7, 11, 12, 15). Post-laminectomy syndrome is a major source of strain in daily activities and of decreased quality of life after back surgery (7). Revision surgery for epidural fibrosis has been associated with complications, such as dural tears, nerve root injuries and epidural bleeding from granulation tissue. Unfortunately, reoperation has been largely unsuccessful (4).

Biological and non-biological materials for the prevention of epidural fibrosis have been studied in animals (5, 10, 13, 16, 23). Some of these experimental materials, such as spinal membrane adhesion barrier gel, have been frequently used in routine neurosurgical practice (5, 10, 12, 13, 18). However, these materials have also generally been associated with a higher cost and with repeated surgery (7, 19).

Several growth factors and cytokines that are partly released from inflammatory cells infiltrating affected tissues have been suggested to play a central role in the initiation and development of fibrosis. Elevated levels of tumor necrosis factor-alpha (TNFα) participate in the activation of the vascular endothelium, regulation of immune response and metabolism of the connective tissue by modulation of fibroblastic function (17). This inflammatory process is a prerequisite for wound repair and scar formation. However, although this response has obvious beneficial aspects, persistent or excessive inflammation can cause later side effects. Etanercept (TNFR:Fc), a fusion protein of TNF receptor (TNFR) type 2 and the Fc portion of human immunoglobulin G (IgG), specifically binds to TNFα, potentially neutralizing its activity (20). Etanercept is effective and has been widely used in the treatment of inflammatory diseases, such as rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis (24). It has been suggested that anti-TNFα agents might also be efficient in the treatment of scleroderma and idiopathic pulmonary fibrosis (17, 21, 24). Some experimental studies have also shown that etanercept was very effective in at reducing dermal sclerosis, collagen accumulation and ventricular remodeling after myocardial infarction and related fibrosis (17).

In the current literature, there is no evidence that the effects of etanercept have been investigated for the prevention of epidural fibrosis formation after lumbar laminectomy. The purpose of this study was to investigate the effects of topical application of etanercept in the prevention of epidural fibrosis in a rat model of simple laminectomy.

MATERIAL and METHODS

Animals

Adult female Wistar rats weighing 250-350 g were used in this study. All the experimental procedures used were approved by the ethical committee of Abant Izzet Baysal University (No: 2013/02), Bolu, Turkey; to minimize the discomfort of the animals during surgery and in recovery.

Surgical Procedure and Sample Preparation

The surgical procedure was performed under general anesthesia induced by intraperitoneal (i.p.) xylazine (10 mg/kg; Bayer, Istanbul, Turkey) and ketamine hydrochloride (60 mg/kg; Parke-Davis, Istanbul, Turkey). After the lower back of the each rat was shaved, the surgical site was sterilized with povidone. A longitudinal midline skin incision was created from L2 to L5. The lumbosacral fascia was incised and the paraspinous muscles dissected subperiosteally to expose laminae L3-L5. Total L3-L5 laminectomy and flavectomy were performed, and 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 the 3 groups, with 8 rats in each group. After treatment, the wounds were closed in anatomical layers using the same suture material (Prolen polypropylene sutures, Ethicon, Ethicon Endo-Surgery, Inc., Cincinnati, Ohio, USA) in each animal. There were no complications or adverse effects due to etanercept. The animals were sacrificed on the 30th postoperative day using a lethal dose (60 mg/kg) of pentobarbital (IE Ulagay, Istanbul, Turkey). The vertebral columns of the rats were removed en bloc between T10 and L5. The specimens were then placed in 10% buffered formalin.

Experimental Groups

Group 1: Control (n=8); only laminectomy was performed; no treatment was administered.

Group 2: Etanercept (Enbrel, Amgen and Pfizer, Immunex Corporation, Thousand Oaks, CA 913220, USA) (n=8); 300 µg/kg etanercept was applied with a spongostan (Ethicon Endo-Surgery, Inc.) soaked with 0.5 ml of the solution and left on the dura mater.

Group 3: Spongostan (n=8); a spongostan was soaked with a 0.5 ml of saline solution and was left on the dura mater.

Evaluation of Epidural Fibrosis

The specimens were decalcified with ethylenediamine tetraacetic acid (EDTA) (R&D Systems Inc., Minneapolis, MN, USA). They were then dehydrated and embedded in paraffin, after complete decalcification. Sections of 10 µm were obtained on the axial plane and were stained with Masson's trichrome. The sections were examined using a Nikon eclipse 80i microscope and were photographed using a Nikon DS-Fi1Camera. All the laminectomy sections were evaluated in a blinded manner by one pathologist, who analyzed the dura thickness, density of fibrosis, and arachnoid 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 100x.

As described by Cemil et al., the dura mater thickness was measured at 3 points. The first sample was harvested from the midpoint of the laminectomy defect, the second sample was obtained 2 mm from the right side of the first sample, and the third sample was obtained 2 mm from the left side of the first sample (7). Epidural fibrosis was graded based on the scheme devised by He et al. (11). Mean values were used for statistical evaluation.

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 less than two-thirds of the laminectomy defect.

Grade 3: Scar tissue adherence was large, affecting more than two-thirds of the laminectomy defect, or the adherence extended to the nerve roots. The presence of arachnoidal involvement was also noted.

Statistical Analysis

Data analysis was performed using SPSS, version 11.5 for Windows (SPSS Inc., Chicago, IL, USA). Descriptive statistics for ordinal variables are represented as 25th percentile-75th percentile, and categorical variables are represented as numbers of rats and percentages (%). The Kruskal-Wallis test was used for the determination of statistical significance between groups regarding the density of epidural fibrosis. If the Kruskal-Wallis test was statistically significant, Conover's nonparametric multiple comparison test was used to determine the situations that caused the differences. The presence of arachnoidal involvement was statistically analyzed using a likelihood ratio test. Statistically significant p-values were determined as < 0.05.

RESULTS

There was no mortality related to the procedure, and all the animals were ambulatory at the time of sacrifice. No wound infection, erythema, hematoma or cerebrospinal fluid leakage was observed. The results of He et al. grading of the epidural fibrosis are shown in Figure 1. The statistical difference was significant when dura mater thickness was compared between the treatment groups and the control group ($p<0.001$). Furthermore, epidural fibrosis in the etanercept group was significantly less than in the spongostan group ($p<0.001$) (Table I). In the axial sections stained with Masson's trichrome, 75% of the rats in the control group showed grade 3 epidural fibrosis (Figure 2). Grade 1, grade 2 and grade 3 epidural fibrosis was observed in 12.5%, 50% and 37.5% of the rats in the etanercept group, respectively (Figure 3); 37.5%, 50% and 12.5% of the rats in the spongostan group showed grade 1, grade 2 and grade 3 epidural fibrosis, respectively (Figure 4). The results of the epidural fibrosis grades are shown in Table II. The arachnoidal involvement was found to be significantly different between the treatment groups and the control group ($p<0.05$). However, there was no significant difference between the etanercept group and the spongostan group ($p>0.05$). The results of arachnoidal involvement are shown in Table III.

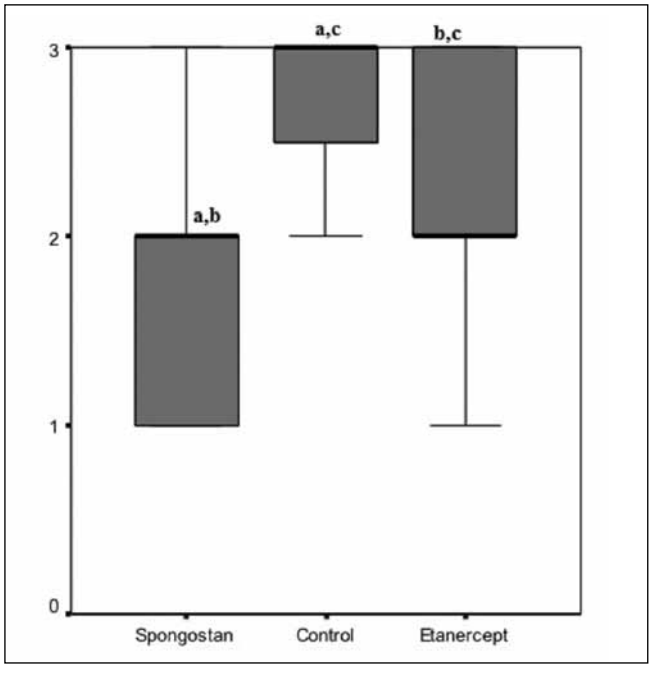

Figure 1: Box-plot graph demonstrating the differences in epidural fibrosis grading among the spongostan, control and etanercept groups. Kruskal-Wallis test, **a:** The difference between the spongostan group and the control group was statistically significant ($p<0,001$); **b:** The difference between the spongostan group and the decorin group was statistically significant ($p<0.001$); **c:** The difference between the control group and the etanercept group was statistically significant ($p<0.001$).

Table I: The Descriptive Table Shows the Histopathological Grades of the Experimental Groups

Groups	Epidural Fibrosis Grade *
Spongostan	2 (1-2) [a, b]
Control	3 (2,5-3) [a, c]
Etanercept	2 (2-3) [b, c]
p-value **	0,025

Data were represented as median 25-75 percentile, ** Kruskal-Wallis test, **a: The difference between the spongostan group and the control group was statistically significant ($p<0,001$), **b:** The difference between spongostan group and the etanercept group was statistically significant ($p<0,001$), **c:** The difference between the control and the etanercept group was statistically significant ($p<0,001$).*

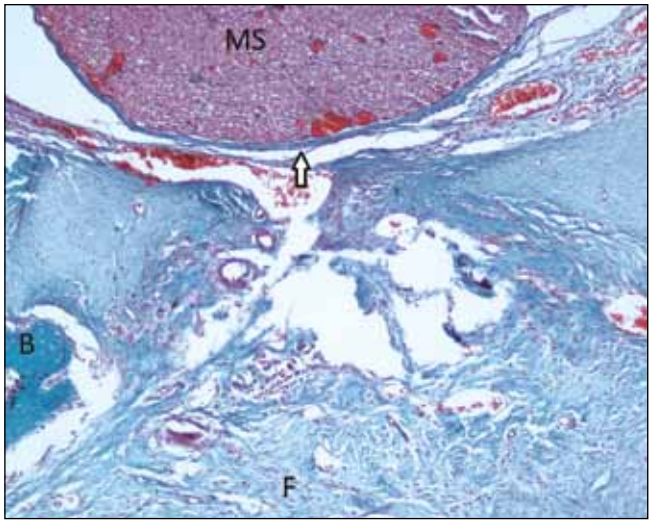

Figure 2: Photomicrograph showing Grade 3 fibrosis, as observed in the control group. Epidural fibrosis (F) completely covered the laminectomy defect and adhered to the underlying dura mater (arrow). Direct contact was evident between the epidural fibrosis tissue and the medulla spinalis (MS). B=Bone. Masson's trichrome, original magnification 100x.

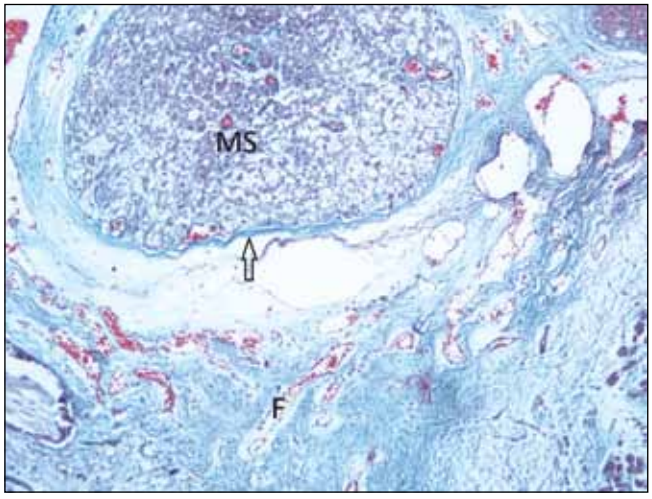

Figure 3: Photomicrograph showing Grade 1 fibrosis, as observed in the etanercept group. Only thin epidural fibrosis (F) adhering to the underlying dura mater (arrow) was demonstrated. No direct contact between the underlying medulla spinalis (MS) and the epidural fibrosis (F) tissue was evident. B=Bone. Masson's trichrome, original magnification 100x.

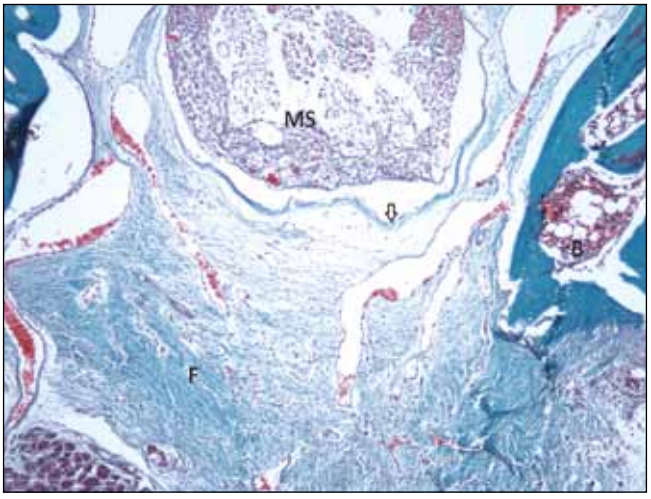

Figure 4: Photomicrograph showing Grade 2 fibrosis, as observed in the spongostan group. Epidural fibrosis (F) covered less than 2/3 of the laminectomy defect and adhered to the underlying dura mater (arrow). B=Bone. Masson's trichrome, original magnification 100x.

Table II: Histopathological Results of Epidural Fibrosis Grades

Grade	Spongostan	Control	Etanercept
I	3 (37,5%)	-	1 (12,5%)
II	4 (50,0%)	2 (25,0%)	4 (50,0%)
III	1 (12,5%)	6 (75,0%)	3 (37,5%)
Total	8 (100,0%)	8 (100,0%)	8 (100,0%)

Table III: Histological Results of Arachnoidal Involvement

Groups	Arachnoidal involvement *
Spongostan	3/8 (%37,5)
Control	1/8 (%12,5)
Etanercept	1/8 (%12,5)
p-value**	0,529

***Data were represented number of rats and percentage (%).* ** *Likelihood ratio test.*

DISCUSSION

Post-laminectomy epidural fibrosis is a well-known entity, and individual variability in the degree of scar formation, postoperative hematoma, postoperative infection, laminectomy techniques, amount of bone removed, anatomic region within the spinal column and race play important roles in the pathogenesis of this condition (15). TNFα inhibits fibrillar collagen gene expression and stimulates cytokines and interstitial collagenase (MMP1 and MMP13) gene expression. TNFα is potent activator of the intracellular signaling molecules cJun N-terminal kinase (JNK or SAPK), p38 MAPK and NF-KB, which regulate expression of type 1 collagen alpha 1, interstitial collagenase and cytokines, respectively. TNFα increases the release of endothelin-1 and the expression of adhesion molecules, such as E-selectin, ICAM-1 and VCAM-1, from endothelial cells. Additionally, it stimulates the proliferation of fibroblasts via TGF-β1 (3). When tissue damage occurs, TGF-β1 is excreted due to degranulation, and it acts as a chemoattractant for inflammatory cells and thrombocytes and stimulates fibroblasts, which are responsible for fibrosis. The key factor for the formation of the fibrosis is fibroblast migration into surgical area (6). Fibroblasts originate from perivertebral muscles and/or are carried by blood into the surgical area. Therefore, prevention of the migration of fibroblasts from the perispinal muscles or from hematoma related to surgery could prevent or reduce epidural fibrosis formation. Various biological and synthetic materials have been investigated for the prevention of scar formation (1, 7, 9, 12, 14, 15, 22).

Some studies have found that the crucial role of TNFα in collagen synthesis and fibrosis could be antagonized and/or reduced by the action of certain agents. Etanercept might improve fibrosis not only by its direct effects but also by decreasing TGF-β levels (25). Etanercept can also be used in scleroderma and in pulmonary and vascular disorders to reduce tissue fibrosis, as well as in the treatment of inflammatory diseases, such as rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis (17). According to the literature mentioned above, it has been hypothesized that etanercept might be helpful in the treatment of post-laminectomy epidural fibrosis. In our study, the dose of etanercept was 300 μg/kg, based on published data (17).

Histopathological evaluation was performed using Masson's trichrome staining. Masson's trichrome is a three-color staining protocol used in histology. Most recipes produce red muscle fibers, blue or green collagen and bone, light

red or pink cytoplasm and dark brown to black cell nuclei. The purpose of the Masson's trichrome stain is primarily to demonstrate the presence of collagen and muscle. It is also used to identify increases in collagenous tissue or to indicate fibrotic changes in cirrhosis of the liver, renal disease, and pulmonary disease and in wound healing processes (2). In our study, fibrosis after simple laminectomy was nicely illustrated in all the rats, and we confirmed using Masson's trichrome staining that etanercept had an anti-fibrotic effect during the wound healing process. Microscopic analysis of the tissue demonstrated no evidence of infection or chronic inflammation related to the surgical procedure. The formation of dense epidural scar tissue and significant adhesion were shown in all the control group rats. The epidural fibrosis grades were significantly lower in the etanercept-treated rats, compared the control group. Furthermore, etanercept was more effective in the prevention of epidural fibrosis than spongostan and this result was statistically significant. The comparison of arachnoidal involvement showed no statistically significant difference between the groups (p=0.529). However, in the etanercept-treated group, only one of the 8 rats showed arachnoidal involvement, whereas 3 of the 8 rats showed arachnoidal involvement in the control group. In the current study, the possible anti-fibrotic mechanism of etanercept not only inhibited TNFα directly but also decreased TGF-β levels indirectly.

In conclusion, our study demonstrated that topical application of etanercept could effectively reduce the formation of epidural fibrosis in a rat model of simple laminectomy. Further research is necessary to determine the optimal dosage and usage of etanercept.

REFERENCES

1. Aydincak O, Yilmaz MB, Emmez H, Kurt G, Sepici A, Memis L, Baykaner K: The effect of temozolomide on the prevention of epidural fibrosis developing after lumbar laminectomy in rats. Turk Neurosurg 22(6): 706-711, 2012
2. Bancroft J, Gamble M: Theory and Practice of Histological Techniques, 6th ed. London: Churchill-Livingstone, 2008
3. Battegay EJ, Raines EW, Colbert T, Ross R: TNF-alpha stimulation of fibroblast proliferation. Dependence on platelet-derived growth factor (PDGF) secretion and alteration of PDGF receptor expression. J Immunol 154(11): 6040-6047, 1995
4. Benoist M, Ficat C, Baraf P, Cauchoix J: Postoperative lumbar epiduro-arachnoiditis. Diagnostic and therapeutic aspects. Spine (Phila Pa 1976) 5(5): 432-436, 1980
5. Bora H, Aykol SV, Akyurek N, Akmansu M, Ataoglu O: Inhibition of epidural scar tissue formation after spinal surgery: External irradiation vs. spinal membrane application. Int J Radiat Oncol Biol Phys 51(2): 507-513, 2001
6. Border W, Noble N: Transforming growth factor beta in tissue fibrosis. N Engl J Med 11:1286–1292, 1994
7. Cemil B, Tun K, Kaptanoglu E, Kaymaz F, Cevirgen B, Comert A, Tekdemir I: Use of pimecrolimus to prevent epidural fibrosis in a postlaminectomy rat model. J Neurosurg Spine 11(6): 758-763, 2009
8. Dogulu F, Kurt G, Emmez H, Erdem O, Memis L, Baykaner K, Ceviker N: Topical mitomycin C-induced inhibition of postlaminectomy peridural fibrosis in rabbits. J Neurosurg 99(1): 76-79, 2003
9. Einhaus SL, Robertson JT, Dohan FC Jr, Wujek JR, Ahmad S: Reduction of peridural fibrosis after lumbar laminotomy and discectomy in dogs by a resorbable gel (ADCON-L). Spine (Phila Pa 1976) 22(13): 1440-1461,1997
10. Gill GG, Scheck M, Kelley ET, Rodrigo JJ: Pedicle fat grafts for the prevention of scar in low-back surgery. A preliminary report on the first 92 cases. Spine (Phila Pa 1976) 10(7): 662-667, 1985
11. He Y, Revel M, Loty B: A quantitative model of post-laminectomy scar formation. Effects of a nonsteroidal anti-inflammatory drug. Spine (Phila Pa 1976) 20(5):557-563, 1995
12. Ismailoglu O, Albayrak B, Gulsen I, Tanriover G, Demir N: Topical application of tacrolimus prevents epidural fibrosis in a rat postlaminectomy model: Histopathological and ultrastructural analysis. Turk Neurosurg 21(4): 630-633, 2011
13. Jacobs RR, McClain O, Neff J: Control of postlaminectomy scar formation: An experimental and clinical study. Spine (Phila Pa 1976) 5(3):223-229, 1980
14. Karatay M, Erdem Y, Koktekir E, Erkoc YS, Caydere M, Bayar MA: The effect of bevacizumab on spinal epidural fibrosis in a postlaminectomy rat model. Turk Neurosurg 22(6): 753-757, 2012
15. Kasimcan MO, Bakar B, Aktaş S, Alhan A, Yilmaz M: Effectiveness of the biophysical barriers on the peridural fibrosis of a postlaminectomy rat model: An experimental research. Injury 42(8): 778-781, 2011
16. Kitano T, Zerwekh JE, Edwards ML, Usui Y, Allen MD: Viscous carboxymethylcellulose in the prevention of epidural scar formation. Spine (Phila Pa 1976) 16(7): 820-823, 1991
17. Koca SS, Isik A, Ozercan IH, Ustundag B, Evren B, Metin K: Effectiveness of etanercept in bleomycin-induced experimental scleroderma. Rheumatology (Oxford) 47(2): 172-175, 2008
18. Kurt G, Aytar MH, Doğulu F, Cemil B, Erdem O, Baykaner MK, Ceviker N: A comparison of the local effectiveness of mitomycin C, aprotinin, and Adcon-L in experimental peridural fibrosis. Surg Neurol 70(6): 608-613, 2008
19. Lo H, Frederickson RC: Use of ADCON in neurosurgery: Preclinical review. Neurol Res 21(1): 27-32, 1999
20. Nedwin GE, Naylor SL, Sakaguchi AY, Smith D, Jarrett-Nedwin J, Pennica D, Goeddel DV, Gray PW: Human lymphotoxin and tumor necrosis factor genes: Structure, homology and chromosomal localization. Nucleic Acids Res 13(17): 6361–6373, 1985
21. Raghu G, Fatenejad S, McDermott L: Efficacy and safety of etanercept in patients with idiopathic pulmonary fibrosis (IPF). Eur Respir J 28:767, 2006

22. Savran M, Bekar A, Cansev M, Tolunay S, Ulus IH, Taskapilioglu MO: Prevention of epidural fibrosis in rats by local or systemic administration of citicoline. Turk Neurosurg 22(5): 634-640, 2012
23. Songer MN, Ghosh L, Spencer DL: Effects of sodium hyaluronate on peridural fibrosis after lumbar laminotomy and discectomy. Spine (Phila Pa 1976) 15(6): 550-554, 1990
24. Tutuncu Z, Morgan GJ Jr, Kavanaugh A: Anti-TNF therapy for other inflammatory conditions. Clin Exp Rheumatol 20(6): 146-151, 2002
25. Yamamoto T, Takagawa S, Katayama I, Nishioka K: Anti-sclerotic effect of transforming growth factor-beta antibody in a mouse model of bleomycin-induced scleroderma. Clin Immunol 92(1): 6-13, 1999