DOI: 10.5137/1019-5149.JTN.18460-16.1
Received: 18.06.2016 / Accepted: 17.10.2016
Published Online: 15.11.2016

Turk Neurosurg 28(1):111-117, 2018

Original Investigation

Effect of Etanercept on the Formation of Epidural Fibrosis in an Experimental Model

Zahir KIZILAY[1], Nesibe KAHRAMAN CETIN[2], Abdullah TOPCU[1], Ozgur ISMAILOGLU[3], Imran KURT OMURLU[4], Serdar AKTAS[5], Ali YILMAZ[1]

[1]Adnan Menderes University, School of Medicine, Department of Neurosurgery, Aydin, Turkey
[2]Adnan Menderes University, School of Medicine, Department of Pathology, Aydin, Turkey
[3]Suleyman Demirel University, School of Medicine, Department of Neurosurgery, Isparta, Turkey
[4]Adnan Menderes University, School of Medicine, Department of Biostatistics, Aydin, Turkey
[5]Adnan Menderes University, School of Veterinary Medicine, Department of Pharmacology and Toxicology, Aydin, Turkey

ABSTRACT

AIM: To investigate the effects of local and systemic administration of etanercept on the formation of epidural fibrosis.

MATERIAL and METHODS: Twenty-eight rats were randomly divided into four equal groups (Control, Spongostan™, Local etanercept and Systemic etanercept) and laminectomy was performed between T11 and L1 in all rats. Spongostan™ was soaked with saline (0.1 mg/kg), local etanercept (300 µg/kg) was applied with Spongostan™ and systemic etanercept (300 µg/kg/week) was applied subcutaneously. Four weeks later, the vertebral column from T9 to L3, including the paraspinal muscles and epidural scar tissue, was removed en bloc, and epidural fibrosis and arachnoidal involvement were graded and evaluated histopathologically.

RESULTS: The grading of epidural fibrosis was statistically significantly lower in systemic and local administration of etanercept groups compared to the control group ($p < 0.005$), but systemic etanercept administration was more effective.

CONCLUSION: Systemic administration of etanercept can be effective in reducing epidural fibrosis in rats after laminectomy.

KEYWORDS: Laminectomy, Epidural fibrosis, Failed back surgery, Etanercept, Rat

INTRODUCTION

Epidural fibrosis (EF) is defined as exaggerated tissue fibrosis in the epidural space after spinal surgery (34). Exaggerated tissue fibrosis may lead to the recurrence of complaints in the postoperative period by causing excessive adhesion in the dura mater and nerve roots. This clinical condition is called failed back surgery syndrome (FBSS)(20,34,35). FBSS was reported to develop in 8–40% of patients who undergo lumbar laminectomy (3). The clinical significance of this syndrome is to increase the probability of complications such as dura mater and nerve injuries and epidural haematoma when these patients are operated on again (6,14,32). Moreover, this exaggerated response occurs after each surgical procedure in patients who develop these clinical conditions and so the success rate of treatment decreases after each surgical procedure (23).

For these reasons, the prevention of epidural scar tissue formation has been a common target in spinal surgery and so it has been the main topic of many studies. In the literature, many studies have been performed to prevent EF. In these studies, many agents were used but no effective treatment option was found (10,12,19,30,35).

When it is considered that EF is developed after an inflammation process, there are many cytokines and growth factors that may be effective in this process, and also there are many

Corresponding author: Zahir KIZILAY
E-mail: zahir.kizilay@adu.edu.tr

cells that synthesize and transfer these substances to the medium. It can be said that all of these may be effective on the formation of EF. Tumour necrosis factor-alpha (TNF-α) is one of these cytokines and a pro-inflammatory cytokine. It is synthesized and displays an effect in immune and non-immune cells such as macrophages, T cells, fibroblasts, mast cells and smooth muscle cells (31). Therefore, a decrease in TNF-α synthesis or its blockade at the receptor level can reduce the formation of EF.

Etanercept, which has been developed to reduce the effectiveness of TNF-α, is a fusion protein of type II TNF receptors. Moreover, it neutralizes the effectiveness of TNF-α receptors by specifically binding to them (5,22,32). Etanercept has been shown to be effective in the treatment of rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis and scleroderma (5,13,15,27). In addition to these, it was reported in an experimental study that local administration of etanercept reduced EF (32).

The purpose of this study was to investigate its effects on the formation of EF after local and systemic administration of etanercept, which was previously reported to inhibit the formation of EF in an EF model in rats.

MATERIAL and METHODS

Animals

Adult Wistar albino rats weighing approximately 250–300 g were used in the study. All experimental protocols conducted on the animals were consistent with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and approved by the Local Ethics Committee on Animal Care (Adnan Menderes University, approval number: 64583101/2016/62, approval date: 25 February 2016).

Experimental Groups

Group 1: Control (C), (n=7); only T12 total laminectomy was performed; no treatment was administered.

Group 2: Spongostan™ (S), (n=7); T12 total laminectomy was performed and distilled water was applied with Spongostan™ on the laminectomy area.

Group 3: Local etanercept (LE), (n=7); T12 total laminectomy was performed and 300 µg/kg etanercept (Enbrel, Amgen and Pfizer, Immunex Corporation, Thousand Oaks, CA9132020, USA) was applied with Spongostan™ on the laminectomy area.

Group 4: Systemic etanercept (SE), (n=7); T12 total laminectomy was performed, and 300 µg/kg/week etanercept was given subcutaneously for 4 weeks.

Surgical Procedure and Sample Preparation

The rats were kept between 22 and 25 °C. Humidity was at an average level and the light-dark cycle was adjusted to 12 h light-12 h dark. The rats had access to food and water ad libitum. Intraperitoneal injection of 10 mg/kg xylazine (Rompun, Bayer, Turkey) and 50 mg/kg ketamine (Ketalar, Parke-Davis, Turkey) was used to anaesthetize the rats while they breathed spontaneously. The body temperatures of the rats were kept at 37 °C by placing them on a heating pad, and they were kept in the prone position. The lower-back parts of the rats were shaved, and the operational area was sterilized with povidone. The same surgeon performed the whole procedure. An incision was taken through the skin as a longitudinal midline over the T10–L2 levels. The thoracolumbar fascia was incised and the paravertebral muscles were dissected in a subperiosteal manner. The T10–L2 laminae were exposed after this procedure. A total laminectomy was performed at the T12 level, then the ligamentum flavum and epidural fat tissues were removed from the surgical site. The dura mater was fully visible at this time and was left intact. Cotton pads were used to achieve haemostasis. The wounds were closed in anatomical layers with 4-0 polypropylene suture after administration of the topical agent. The animals were sacrificed on the 30th postoperative day. The vertebral columns of the rats were removed en bloc between T9 and L3.

Histopathological Evaluation

The vertebral column from T9 to L3, including the paraspinal muscles and epidural scar tissue, was removed en bloc and placed in 10% neutral buffered formalin solution for fixation. The specimens were decalcified in 10% formic acid for approximately 7 days. After decalcification, tissue samples were taken from the laminectomy areas. They were washed with tap water and after that were passed through routine tissue processing. Then, the specimens were embedded in paraffin. Four-micron axial sections of the samples were cut and stained with haematoxylin–eosin and Masson's trichrome stains. All histopathologic sections were evaluated by a pathologist blinded to the treatment groups. The same pathologist also analysed arachnoid involvement and EF grade.

Fibrous tissue was examined in haematoxylin–eosin and Masson's trichrome-stained sections using an Olympus BX52 microscope and photographed using an Olympus DP 25 camera. The extent of the EF along with the dura mater was evaluated according to the scale described by He et al.(9). The presence of arachnoidal involvement was also noted.

Grade 0: The 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.

Statistical Analysis

Data analysis was performed using SPSS. Descriptive statistics for ordinal variables are represented as 25th percentile-75th percentile. The Kruskal–Wallis test was used for the determination of statistical significance between groups regarding the density of EF. For arachnoidal involvement, we used Pearson's chi-square by computing the exact p-value

and the results were shown as frequencies. Statistically significant p-values were determined as < 0.05.

RESULTS

Complications Associated with Wound Recovery and Medical Practice

No adverse effects of etanercept were seen in the wound area and the peripheral tissues. In addition, no infection, cerebrospinal fluid collection, rash or haematoma secondary to laminectomy was seen in the wound area. No paraplegia or paraparesis was observed in the groups.

Histopathological Evaluation

The groups were histopathologically graded according to the He et al.'s (9) scheme. Grade 2 and 3 EF was observed in two and five rats, respectively, in Group 1 (Figure 1A). Grade 1, 2 and 3 was observed in three, three and one rats, respectively, in Group 2 (Figure 1B). Grade 1 and 2 EF was observed in five and two rats, respectively, in Group 3 (Figure 1C). Grade 0 and 1 was observed in one and six rats, respectively, in Group 4 (Figure 1D). These results showed that systemic and local administration of etanercept was superior to total laminectomy in reducing EF ($p<0.001$, $p=0.016$)(Table I). When the groups were compared with each other, there was no statistically significant difference between the local etanercept and systemic etanercept groups ($p>0.05$) (Figure 2).

The groups were evaluated in terms of arachnoid involvement. While it was found in four rats in Group 1, one rat in Group 2 and one rat in Group 3, it was not seen in Group 4. These results showed that the local and systemic administration of etanercept was effective in reducing arachnoid involvement compared to only total laminectomy but there was no statistically significant difference between the groups (Table I, Figure 3).

DISCUSSION

EF occurs as a result of the natural inflammatory process. However, EF that occurs at the end of the natural healing process leads to the retraction of the dura mater and nerve roots by causing adhesions around them, and it also causes unwanted effects such as the emergence of complaints before reoperation. Therefore, EF is a significant problem in the long term. Many authors believe that the best way to protect against unwanted effects of EF is to prevent the formation of EF (28). Therefore, the literature contains many studies performed to prevent EF. In these studies, autologous fat grafts, Silastic, gelfoam, recombinant plasminogen activator, polyvinyl alcohol hydrogel membrane and carbohydrate polymers were used (8,11,16-18). Despite these agents, an effective treatment was not developed (35).

When it is considered that EF occurs as a result of the natural inflammatory process, TNF-α and other cytokine levels increase in response to the stress that has developed after injury. TNF-α is a pro-inflammatory cytokine and is presented as a transmembrane protein or a soluble cytokine in many immune and non-immune cells, especially activated macrophages and others such as T cells, mast cells, granulocytes, natural killer cells, fibroblasts, neurons, keratinocytes and smooth muscle cells (2,7,31). TNF-α interacts with TNF receptor-1 (TNFR1) and TNF receptor-2 (TNFR2) which are structurally similar but functionally different from each other. TNFR1 is expressed by all cells except red blood cells and TNFR2 is generally expressed by endothelial cells and immune cells (2). TNF-α increases the expression of adhesion molecules, such as endothelin-1, E-selectin, intercellular adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM-1) in endothelial cells (1). In addition, it has been reported to increase the proliferation and differentiation of fibroblasts and the formation of α-smooth muscle actin (α-SMA) and extracellular matrix via transforming growth factor-beta 1 (TGF-β1) (1,26). In studies related to EF, TGF-β1 was reported to play a key role in its formation (36).

TNF-α has been determined to have these effects on immune and non-immune cells and so anti-TNF-α agents have been thought to be effective in the treatment of diseases such as rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis and scleroderma. This situation has been proven in some studies (5,13,15,27). Etanercept (an anti-TNF-α agent) is a fusion protein of TNFR2 receptors and binds as a competitive inhibitor to two of three receptor-binding sites on the TNF trimer and so protects cell membrane receptors from the effects of TNF (4,18). Etanercept has been used in experimental studies (15,32). When studying the efficacy of anti-TNF-α agents on the formation of EF, Turkoglu et al. reported that when 300 μg/kg etanercept was administered locally, it had a statistically significant effect in reducing EF compared to the control group but there was no statistically significant difference between the two groups in terms of arachnoid involvement. The researchers reported that etanercept could display this effect by reducing TGF-β levels in addition to directly blocking TNF-α (32).

In our study, the dose of etanercept was adjusted based on the study of Turkoglu et al. (32), and systemic administration of etanercept was investigated to be effective in the formation of EF. The results of our study showed that the systemic and local etanercept administrations were significantly more effective in preventing EF when compared with the control group. There could be many reasons for this effect. In studies in the literature examining the process of inflammation in wound healing, it was reported that TNF-α increased the expression of adhesion molecules, such as endothelin-1, E-selectin, ICAM-1 and VCAM-1 in endothelial cells and also the proliferation and differentiation of fibroblasts and the formation of α-SMA and extracellular matrix via TGF-β1. However, in studies related to EF models, the proliferation and differentiation of fibroblasts and the formation of α-SMA and extracellular matrix were reported to play an important role, especially in the formation of EF. Therefore, inhibition of TNF-α appears to be an effective method of preventing EF. In studies related to anti-TNF-α agents, the use of anti-TNF-α agent was shown to reduce the TGF-β1 level in serum and the number of α-SMA positive cells and the amount of OH-proline in tissue (15). Moreover, in studies on the efficacy of anti-TNF agents on the inflammation process, they were reported to reduce

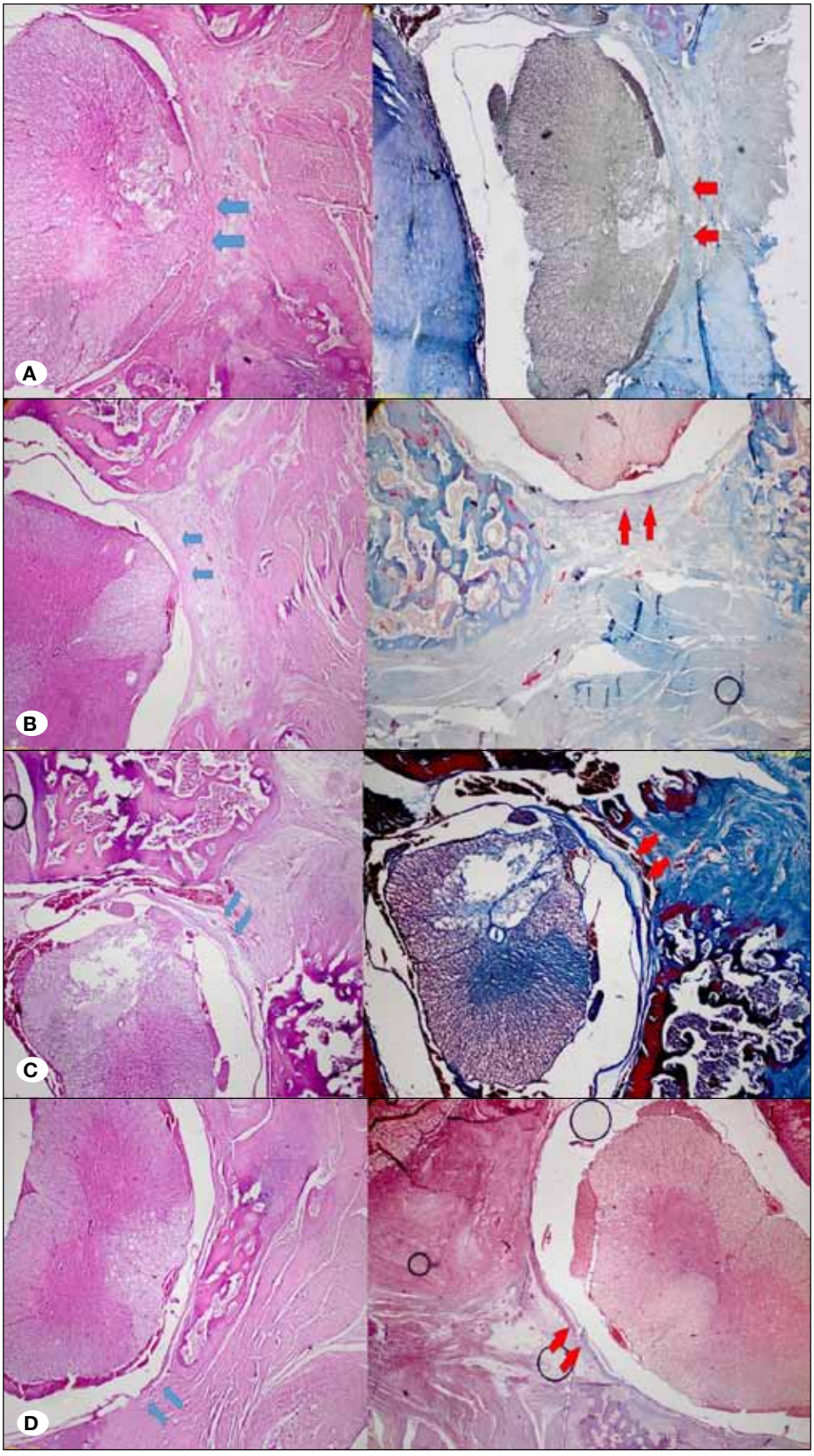

Figure 1: Haematoxylin–eosin (´40 magnification) and Masson's trichrome (´40 magnification) staining of epidural fibrosis in the laminectomy sites in the four groups. **A)** In Group 1, grade 3 fibrosis was observed. Scar tissue completely covered the laminectomy defects and adhered to the underlying dura mater (arrow). Direct contact was evident between the epidural fibrosis tissue and the medulla spinalis. **B)** In Group 2, grade 2 fibrosis was observed. Scar tissue adhered to the underlying dura mater and covered less than two-thirds of the laminectomy sites (arrow). **C)** In Group 3, grade 1 fibrosis was observed. Only thin scar tissue adhered to the underlying dura mater (arrow). **D)** In Group 4, grade 0 fibrosis was observed in one case. The dura mater was free of scar tissue (arrow).

Table I: Groups and Their Pathological Grading according to He et al.'s (9) Scheme and the Numerical Values of Arachnoidal Involvement

	Control	Spongostan™	Local Etanercept	Systemic Etanercept	p value
Histopathological Grade	3 (2-3)*	2 (1-2)	1 (1-2)	1 (1-1)	0.001
Arachnoidal involvement					
Yes	4	1	1	0	0.102
No	3	6	6	7	

**The control group was statistically different from the local (p=0.016) and systemic etanercept (p<0.001) groups.*

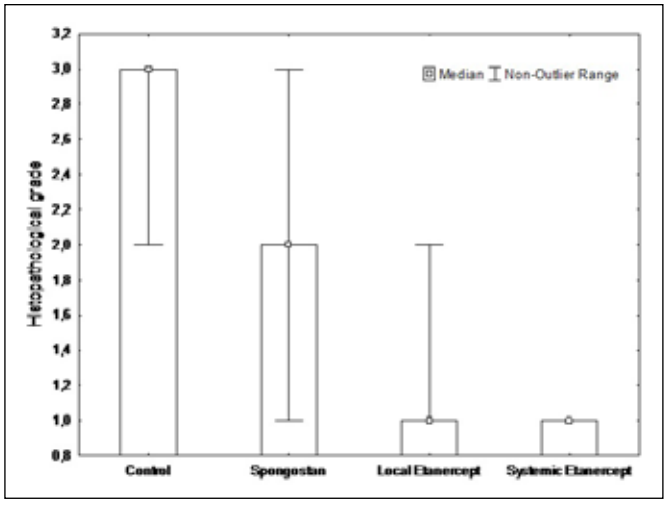

Figure 2: Histopathological assessment of group epidural fibrosis grade.

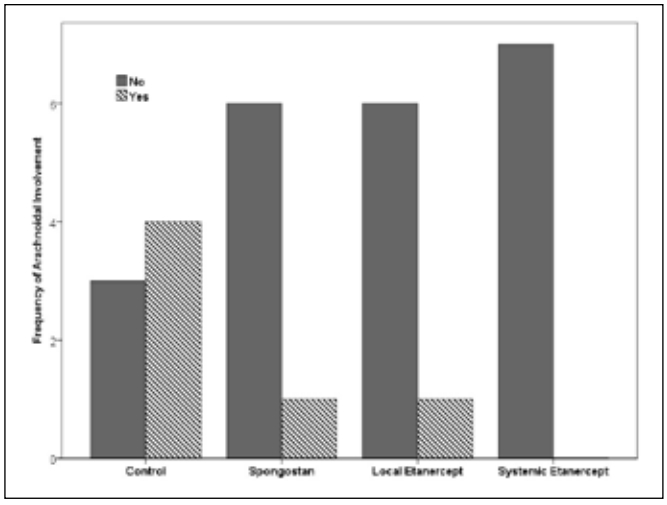

Figure 3: Frequency of arachnoidal involvement of the groups.

the expression of adhesion molecules such as vascular endothelial growth factor (VEGF), VCAM-1, ICAM-1 and E-selectin (24,25,29). When the effects of anti-TNF agents are considered, they seem to be able to influence wound healing phases such as haemostasis, inflammation and proliferation. In our study, when we examined the laminectomy areas in the pathological specimens, it was observed that fibrosis density was decreased in the laminectomy areas, especially during systemic administration of etanercept. When these findings are considered in light of the literature, it is shown that etanercept reduced cell proliferation and fibrosis formation and this situation resulted in a decrease in EF. These findings support that etanercept (an anti-TNF-α agent) can display this effect by inhibiting TNF-α and TGF-β1.

Another result of our study is that there were no statistically significant differences between the local and systemic etanercept administration groups in preventing the formation of EF and arachnoidal involvement. In addition, systemic etanercept administration was more effective in preventing EF compared to local etanercept. There could be many reasons for these findings. The efficacy of etanercept (an anti-TNF agent) was reported to be dose-dependent (5). Moreover, another reason is that it may be related to the time of administration. In the literature, anti-TNF agents were shown to cause a reduction in TNF expression after 2 weeks of use. Moreover, the use of etanercept for 3–12 weeks was reported to cause a reduction in VEGF expression (25,33). Therefore, systemic etanercept administration may be more effective in preventing EF compared to local etanercept, as systemic etanercept was administered for 4 weeks and was applied at four times higher doses compared to the local etanercept group in our study. In contrast to this, use of etanercept for a long period, such as in rheumatoid arthritis patients, may increase delayed spinal infection (21).

There are a few limitations of the study. Firstly, the study had a limited number of rats. Secondly, different doses and durations of etanercept should also be investigated.

■ CONCLUSION

Administration of systemic etanercept is more effective for preventing EF, but there is no difference between local and systemic etanercept administration. As the effect of etanercept depends on dose and time of administration, further studies are needed to determine the optimal dosage and duration for preventing EF.

REFERENCES

1. 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: 6040–6047, 1995
2. Bayrakli F, Balaban H, Ozum U, Duger C, Topaktas S, Kars HZ: Etanercept treatment enhances clinical and neuroelectrophysiological recovery in partial spinal cord injury. Eur Spine J 21(12): 2588-2593, 2012
3. Burton CV, Kirkaldy-Willis WH, Yong-Hing K, Heithoff KB: Causes of failure of surgery on the lumbar spine. Clin Orthop Relat Res 157: 191–199, 1981
4. Chen KB, Uchida K, Nakajima H, Yayama T, Hirai T, Watanabe S, Guerrero AR, Kobayashi S, Ma WY, Liu SY, Baba H: Tumor necrosis factor-α antagonist reduces apoptosis of neurons and oligodendroglia in rat spinal cord injury. Spine (Phila Pa 1976) 36(17): 1350-1358, 2011
5. Cobo-Ibanez T, Martin-Mola E: Etanercept: Long-term clinical experience in rheumatoid arthritis and other arthritis. Expert Opin Pharmacother 8(9): 1373-1397, 2007
6. Cruccu G, Aziz TZ, Garcia-Larrea L, Hansson P, Jensen TS, Lefaucheur JP, Simpson BA, Taylor RS: EFNS guidelines on neurostimulation therapy for neuropathic pain. Eur J Neurol 14: 952–970, 2007
7. Ferguson AR, Christensen RN, Gensel JC, Miller BA, Sun F, Beattie EC, Bresnahan JC, Beattie MS: Cell death after spinal cord injury is exacerbated by rapid TNF alpha-induced trafficking of GluR2-lacking AMPARs to the plasma membrane. J Neurosci 28: 11391–11400, 2008
8. 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 10: 662–667, 1985
9. He Y, Revel M, Loty B: A quantitative model of postlaminectomy scar formation. Effects of a nonsteroidal antiinflammatory drug. Spine (PhilaPa 1976) 20(5): 557–563, 1995
10. Henderson R, Weir B, Davis L, Mielke B, Grace M: Attempted experimental modification of the postlaminectomy membrane by local instillation of recombinant tissue-plasminogen activator gel. Spine (Phila Pa 1976) 18(10): 1268-1272, 1993
11. Hiraizumi Y, Transfeldt EE, Fujimaki E, Nambu M: Application of polyvinyl alcohol hydrogel membrane as anti-adhesive interposition after spinal surgery. Spine (Phila Pa 1976) 20 (21): 2272-2277,1995
12. Kato T, Haro H, Komori H, Shinomiya K: Evaluation of hyaluronic acid sheet for the prevention of postlaminectomy adhesions. Spine J 5: 479–488,2005
13. Katsicas MM, Russo R: Biologic agents in juvenile spondyloarthropathies. Pediatr Rheumatol Online J 14(1):17, 2016
14. Kim SS, Michelsen CB: Revision surgery for failed back surgery syndrome. Spine (Phila Pa 1976) 17: 957-960, 1992
15. 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
16. LaRocca H, Macnab I: The laminectomy membrane: Studies in its evolution, characteristics, effects, and prophylaxis in dogs. J Bone Joint Surg (Br) 56: 545–550, 1974
17. Lawson KL, Malycky JL, Berry JL, Steffee AD: Lamina repair and replacement to control laminectomy membrane formation in dogs. Spine 16: S222–S226, 1991
18. Li C, Wang H, Liu H, Yin J, Cui L, Chen Z: The prevention effect of poly (L-glutamic acid)/chitosan on spinal epidural fibrosis and peridural adhesion in the post-laminectomy rabbit model. Eur Spine J 23(11): 2423-2431, 2014
19. Liu J, Ni B, Zhu L, Yang J, Cao X, Zhou W: Mitomycin C-polyethylene glycol controlled-release film inhibits collagen secretion and induces apoptosis of fibroblasts in the early wound of a postlaminectomy rat model. Spine J 10: 441–447, 2010
20. Ly P, Zhao J, Su W, Liang X, Zhang K: An experimental novel study: Hiperbaric oxygen treatment on reduction of epidural fibrosis via a down-regulation of collagen deposition, IL-6, and TGF-β1. Eur J Orthop Surg Traumatol 25 (Suppl 1): 53-58, 2015
21. Mori S, Tomita Y, Horikawa T, Cho I, Sugimoto M: Delayed spinal infection after laminectomy in a patient with rheumatoid arthritis interruptedly exposed to anti-tumor necrosis factor alpha agents. Clin Rheumatol 27(7): 937-939, 2008
22. 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
23. North RB, Campbell JN, James CS, Conover-Walker MK, Wang H, Piantadosi S, Rybock JD, Long DM: Failed back surgery syndrome: 5-year follow-up in 102 patients undergoing repeated operation. Neurosurgery 28:685-690, 1991
24. Paleolog EM, Hunt M, Elliott MJ, Feldmann M, Maini RN, Woody JN: Deactivation of vascular endothelium by monoclonal antitumor necrosis factor alpha antibody in rheumatoid arthritis. Arthritis Rheum 39: 1082–1091, 1996
25. Paleolog EM, Young S, Stark AC, McCloskey RV, Feldmann M, Maini RN: Modulation of angiogenic vascular endothelial growth factor by tumor necrosis factor alpha and interleukin-1 in rheumatoid arthritis. Arthritis Rheum 41: 1258–1265, 1998
26. Pierce GF, Mustoe TA, Lingelbach J, Masakowski VR, Griffin GL, Senior RM, Deuel TF: Platelet-derived growth factor and transforming growth factor-beta enhance tissue repair activities by unique mechanisms. J Cell Biol 109:429–440, 1989
27. Romero-Mate A, Garcia-Donoso C, Cordoba-Guijarro S: Efficacy and safety of etanercept in psoriasis/psoriatic arthritis: An updated review. Am J Clin Dermatol 8(3):143-155, 2007
28. Sandoval MA, Hernandez-Vaquero D: Preventing peridural fibrosis with nonsteroidal anti-inflammatory drugs. Eur Spine J 17(3): 451-455, 2008
29. Tak PP, Taylor PC, Breedveld FC, Smeets TJ, Daha MR, Kluin PM, Meinders AE, Maini RN: Decrease in cellularity and expression of adhesion molecules by anti-tumor necrosis factor alpha monoclonal antibody treatment in patients with rheumatoid arthritis. Arthritis Rheum 39:1077–1081, 1996
30. Temel SG, Ozturk C, Temiz A, Ersozlu S, Aydinli U: A new material for prevention of epidural fibrosis after laminectomy. J Spinal Disord Tech 19: 270–275, 2006

31. Tracey D, Klareskog L, Sasso EH, Salfeld JG, Tak PP: Tumor necrosis factor antagonist mechanisms of action: A comprehensive review. Pharmacol Ther 117(2): 244-279, 2008
32. Turkoglu E, Tuncer C, Dinc C, Serbes G, Oktay M, Sekerci Z: The effect of etanercept on spinal epidural fibrosis in a postlaminectomy rat model. Turk Neurosurg 24(4):506-511, 2014
33. Ulfgren AK, Andersson U, Engström M, Klareskog L, Maini RN, Taylor PC: Systemic anti-tumor necrosis factor alpha therapy in rheumatoid arthritis down-regulates synovial tumor necrosis factor alpha synthesis. Arthritis Rheum 43: 2391–2396, 2000
34. Zeinalizadeh M, Miri SM, Ardalan FA, Maleki F, Zakeri M, Aghajanzadeh E, Habibi Z: Reduction of epidural fibrosis and dural adhesions after lamina reconstruction by absorbable cement: An experimental study. Spine J 14(1): 113-118, 2014
35. Zhang C, Kong X, Ning G, Liang Z, Qu T, Chen F, Cao D, Wang T, Sharma HS, Feng S: All-trans retinoic acid prevents epidural fibrosis through NF-kB signaling pathway in post-laminectomy rats. Neuropharmacology 79: 275-281, 2014
36. Zhu J, Li Y, Shen W, Qiao C, Ambrosio F, Lavasani M, Nozaki M, Branca MF, Huard J: Relationships between transforming growth factor-beta1, myostatin, and decorin: Implications for skeletal muscle fibrosis. J Biol Chem 282: 25852–25863, 2007