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EXPERIMENTAL STUDY

The role of pegaptanib sodium in the suppression of epidural fibrosis in a postlaminectomy rat model

Sertbas I[1], Yilmaz A[2], Yildirim T[2], Karatay M[3], Celik H[4], Bayar MA[4]

Bartin State Hospital, Neurosurgery, Bartin, Turkey. **mdtimur@hotmail.com**

ABSTRACT

OBJECTIVE: Spinal epidural fibrosis is a clinical condition that develops after laminectomy and can compress the spine. Many agents have been tried for the treatment, but none has entered clinical use at present. Pegaptanib sodium is an antiangiogenetic drug that prevents the development of new vessels and thus adhesion by inhibiting the effect of VEGF.

MATERIAL AND METHOD: 20 Wistar rats were used in this study. The rats were divided into 2 different groups as the control and pegaptanib sodium group. Three levels of laminectomy were performed. Only laminectomy was performed in the control group. A cotton ball soaked with 3 mg/kg Pegaptanib sodium diluted 1: 10 with 0.9 % NaCl was topically applied to the dura in the surgical field for 5 minutes in the pegaptanib sodium group. The rats were sacrificed 3 weeks later and histopathologically examined. The epidural fibrosis was graded.

RESULTS: The epidural fibrosis grade in the pegaptanib sodium was significantly lower than in the control group $c^2 = 11{,}65$; ($p = 0.004$)

CONCLUSION: Pegaptanib sodium blocked the VEGF through its anti-VEGF effect and decreased spinal epidural fibrosis in rats that had undergone laminectomy *(Tab. 2, Fig. 3, Ref. 53)*. Text in PDF *www.elis.sk.*

KEY WORDS: pegaptanib sodium, laminectomy, epidural fibrosis.

Introduction

Epidural fibrosis is the formation of fibrosis tissue in place of other tissues, such as epidural fat tissue, ligament and bone, removed after laminectomy, hemi-laminectomy and other surgical procedures performed for the treatment of disorders compressing the spinal cord or nerve fibres, and is an important cause of back pain (1, 2, 3). The compression of and adhesion to the surrounding nerve tissue and dura mater can lead to clinical signs and symptoms (2, 3). Epidural fibrosis after surgery is a natural result of surgical trauma. The incidence of the epidural fibrosis is between 20 % and 47 % in various series (4). Although epidural fibrosis develops frequently after decompressive surgery, the rate of developing into a clinical disorder is only 1–2 % in these patients (5, 6, 7).

Many agents, such as silastic membrane, carboxymethylcellulose, polylactic acid membrane, glucocorticoids, fibrin glue, spongostan, urokinase, sodium hyalunorate, and adcon-L (anti-adhesion barrier gel), have been used in order to prevent spinal epidural fibrosis (5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15). Vascular Endothelial Growth Factor (VEGF) is a powerful angiogenic cytokine. It also participates directly and actively in VEGF tissue regeneration, fibroblast function, wound healing, and inflammatory reactions (16). VEGF plays a role in the formation of new vessels that cause fibrosis after surgery (17). Pegaptanib sodium has proven efficacy in the treatment of neovascular age-related macular degeneration and prevents angiogenesis by attaching to the VEGF 165 isoform (18, 19). In this study, we investigated the effect of pegaptanib sodium on epidural fibrosis.

[1]Bartin State Hospital, Neurosurgery, Bartin, Turkey, [2]Ordu University School Of Medicine, Neurosurgery, Ordu, Turkey, [3]Gaziosmanpasa Hospital, Neurosurgery, Istanbul, Turkey, and [4]Ankara Training And Research Hospital, Neurosurgery, Ankara, Turkey

Address for correspondence: T. Yildirim, Ordu University Faculty Of Medicine, Neurosurgery Deparment, 52200, Ordu, Turkey.
Phone/Fax: +904522252342

Material and methods

Animals

Totally, 20 male Wistar type, 8–12-month-old rats weighing 200–250 gr were used in this experimental study. The study was conducted at the Experimental Animals Laboratory of Ankara Training and Research Hospital upon receiving the approval of the local ethics committee. Histopathological analyses were performed at the Pathology Department of Ankara Training and Research Hospital.

Surgical procedure and sample preparation

Ketamine hydrochloride (25 mg/kg; Ketalar, Pfizer, Istanbul) and Xylazine (5 mg/kg; Rompun, Bayer, Istanbul) were administered intravascularly for anaesthesia before the procedure. The rats were put in supine position and their backs were shaved. The surgical area was sterilized with povidone iodine (Batticon, Adeka medicine, Istanbul). The surgical field was then covered with sterile drapes. A midline skin incision was performed over the

Tab. 1. Histopathological classification of the epidural fibrosis was in accordance with the He criteria.

Grade 0	No fibrosis affecting the dura mater
Grade I	Fine fibrous bands between fibrous tissue and dura mater
Grade II	Permanent adhesion present in an area less than two thirds of the laminectomy defect
Grade III	Adhesion of fibrosis tissue present in an area more than two thirds of the laminectomy defect and/or fibrous tissue reaches the nerve roots

Tab. 2. Comparison of the histopathology results of the control and treatment groups for epidural fibrosis.

Groups	Stage n (%)			
	stage 0	stage 1	stage 2	stage 3
Control	0 (0.0)	0 (0.0)	3 (30.0)	7 (70.0)
Pegaptanib Sodium	1 (10.0)	6 (60.0)	2 (20.0)	1 (10.0)

$\chi^2 = 11.65$, $p = 0.004$

L1–S1 spinous processes. The paraspinal muscles were dissected by bilateral microdissection. Total laminectomy was performed on the L3, L4 and L5. Epidural fat tissue and ligamentum flavum were cleaned. The dura mater was exposed. A bipolar coagulator was used for hemostasis. No dura or nerve damage occurred. Only laminectomy was performed in the control group. A cotton ball soaked in 3 mg/kg pegaptanib sodium diluted 1:10 with 0.9 % NaCl was topically applied to the dura in the surgical field for 5 minutes in the pegaptanib sodium group. Following hemostasis, the anatomic layers were closed properly one by one. Both the control and the experiment group rats were taken to their cages and provided with routine care. The subjects were kept alive for 3 weeks and then sacrificed by intraperitoneal administration of high-dose (75–100 mg/kg) Thiopental Sodium (Pentothal Sodium, Abbott, Italy). No infection developed in any of the rats. The vertebral column (L3–L4–L5) was removed an bloc.

The block of the vertebral column was fixed in 10 % buffered formaldehyde for 4 days and decalcified with 30 % formic acid for 2 days for histopathology analysis. The amount of the epidural fibrosis at the laminectomy area and its relationship with the dura mater were defined using histopathology classifications and the results were analysed. Three 2 mm-thick transverse spinal cord samples were obtained from the laminectomy region (from the middle, proximal, and distal sections). Following paraffin fixation, 3 micron-thick profiles were taken from the paraffin blocks with a microtome. Hematoxylin and eosin staining was performed for routine histopathological analyses. These preparations were evaluated with a light microscope. Fibrous tissue was defined with the "Zeiss Imager M2" microscope and photographed.

Experimental groups

Group 1: Control (n = 10); only laminectomy was performed, no treatment was given.

Group 2: Pegaptanib sodium (n = 10); A cotton ball soaked in 3 mg/kg pegaptanib sodium (20 he 21) diluted with 0.9 % sodium chloride was topically applied to the dura for 5 minutes.

Evaluation of epidural fibrosis

The epidural fibrosis was graded according to the classification of He et al (16, 20) (Tab. 1).

Statistical analysis

Chi-square analysis (Fisher's Exact test) was used to present the scores of epidural fibrosis depending on the groups (Control and Pegaptanib Sodium). The findings were expressed as the sample size (%). All the computational work was performed by means of SPSS 21.0 V. $p < 0.05$ were accepted as statistically significant.

Results

The treatment and the control groups were compared using the He criteria for microscopic evaluation. No dura or nerve injury was observed in any of the treatment and the control group subjects during the surgical procedure. There were also no complications due to pegaptanib sodium in any of the subjects in the treatment group. Epidural fibrosis was intense in the control group, but less prominent in the treatment group. Stage 2 epidural fibrosis was present in 30 % and stage 3 in 70 % of the control group. Stage 0 was found in 10 %, stage 1 in 60 %, stage 2 in 20 % and stage 3 in 10 % in the pegaptanib sodium group (Tab. 2). Epidural fibrosis extended to the dura from beneath the paraspinal muscles and was adherent. Spaces were found between the dura mater around the medulla spinalis and surrounding muscle layer and nerve roots of the treatment group subjects. Occasional dura mater and bundles extending to the peripheral tissue with a small amount of fibroblast proliferation were observed in these spaces. Comparison of the histopathology results of the control and treatment groups for epidural fibrosis indicated less fibrosis in the treatment group, which was statistically significant $\chi^2 = 11.65$; ($p = 0.004$) (Tab. 2, Figs 1, 2, 3).

Discussion

The reasons of recurrent pain after laminotomy or laminectomy performed during surgery for lumbar disc problems or for other disorders are yet to be explained adequately. The most important etiological factor is thought to be the scar tissue at the epidural region emerging as a natural result of the healing process (21, 22, 23, 24). The scar tissue is reported to create adhesions between the tissues, compress peripheral anatomic structures when prominent, restrict nerve root movement and increase their sensitivity, and cause neural atrophy and axonal degeneration due to scar tissue formation (21, 25, 26, 27). Chemotactic factors emerging with the disruption of erythrocytes and platelets following epidural bleeding and fibroblastic cell migration from the paraspinal muscles are the source of post-laminectomy epidural fibrosis. Adhesions occur due to the fibrous connective tissue hyperplasia. Fibroblasts are important repair cells and try to repair the local vertebral lamina defect following activation by inflammatory cytokines and growth factor (transforming growth factor β and basic fibroblast growth factor). Fibroblasts transform into fibrocytes with the production of collagen fibre. The fibrous connective tissue turns into scar

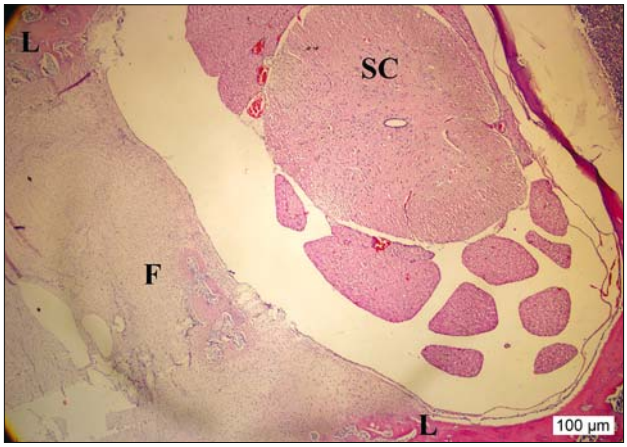

Fig. 1. Stage 3 epidural fibrosis at the control group. SC– Spinal Cord, L– Lamina, F– Fibrosis, Scale bar = 100 µm.

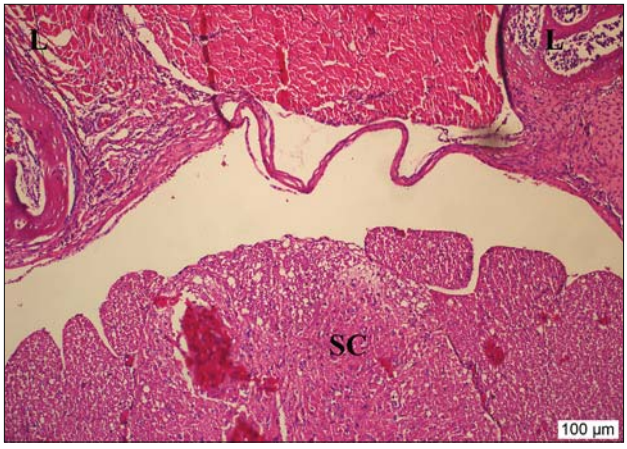

Fig. 2. Stage 0 epidural fibrosis in pegaptanib sodium group. SC– Spinal Cord, L– Lamina, Scale bar = 200 µm.

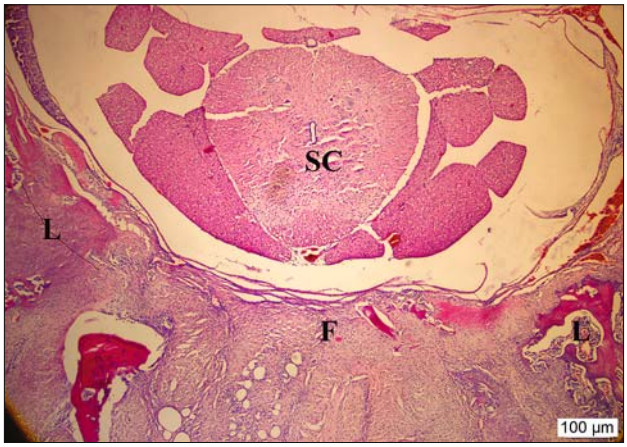

Fig. 3. Stage 1 epidural fibrosis in pegaptanib sodium group. SC– Spinal cord, L– Lamina, F– Fibrosis, Scale bar = 100 µm.

tissue at the same time. Epidural fibrous tissue can adhere to the dura or the nerve roots at the spinal canal, and gradually turn into a laminectomy membrane. This secondary membrane has been reported to cause spinal stenosis, dural compression and restriction of nerve root mobility (4, 28, 29, 30, 31). Repeated surgery to remove the secondary compression caused by epidural fibrosis can increase the fibrosis itself so it is best to try to avoid it in the first operation (32, 33).

A large number of experimental and clinical studies have been conducted to prevent epidural fibrosis. The most commonly used substances are Silastic-Dacron gelatin sponge, animal collagen membranes, Adcon-L, autologous fat graft, omental graft, local cortisone, polyvinyl alcohol, vicryl mesh, viscous solution of sodium hyaluronate, urokinase, triamcinolone, ketoprofen, polylactic acid membrane, polytetrafluoroethylene membrane, and recombinant tissue plasminogen activator as fibrinolytic agent gel. Although successful results are reported in most of these studies, routine clinical use cannot be started as there is still no report of sufficient efficacy consistent with clinical improvement in humans (5, 7, 34, 35). The most commonly used substance to prevent epidural fibrosis is autogenous fat tissue. Epidural fat tissue is histopathologically different from subcutaneous fat tissue. Wolfram et al reported that epidural fat tissue consisted of adipocytes and a small amount of connective tissue in a histopathology study (36). Subcutaneous fat tissue contains more connective tissue. This fat tissue is known to decrease dural adhesions, but has a partial inhibitory effect on epidural fibrosis. Bryant et al (37) concluded that autogenous free fat grafts were well tolerated and also prevented epidural fibrosis tissue from progression to the spinal canal with revascularization in the short- and long-term follow-up of 44 patients.

Braverman et al reported that the success rate was only 30–35 % in cases they operated for epidural fibrosis (38). Epidural fibrosis is known to develop in various degrees in almost all cases after lumbar discectomy. Some investigators have reported that surgical intervention in the epidural space is responsible for the inflammatory reaction that results in the accumulation of collagen deposits caused by fibroblasts coming to the region and that the fibrous tissue may also be the result of surgical complications such as haemorrhage and infection (39, 40).

Benoist et al reported no pathological finding other than marked epidural fibrosis in any of the patients during the second surgery in 38 patients, who did not experience pain relief following lumbar disc hernia surgery, and reported adhesive arachnoiditis in 3 cases (39). A study investigating the aetiology of focal spinal arachnoiditis reported that the nucleus pulposus caused fibrosis in the arachnoid membrane and epidural space, and the reason was inflammation due to the nucleus pulposus leaking into the epidural space. The rate and the persistence of arachnoiditis and neural degeneration have been reported to increase in the presence of foreign bodies, and the occurrence of axonal degeneration of the cauda equina to be more common in the dorsal aspect that faces the scar tissue. Some investigators have reported that cotton fibres remaining after surgery play a role in the aetiology of epidural fibrosis and arachnoiditis (40, 41, 42, 43). The success rate of surgery for epidural fibrotic changes is 30–37 %, while 10–20 % of the cases have been reported to deteriorate (44). North et al (45) reported good results in patients

with mostly radicular pain, but no epidural fibrosis requiring surgical intervention. Epidural fibrosis indicated poor prognosis as demonstrated by such patients undergoing 2–4 surgeries in average with a success rate of 34 %. Fager et al (31) reported improvement in 1 % of the re-operated cases and no change in 34 %, while 25 % got worse afterwards. Jayson et al (46) reported good results in patients operated for recurrent disc hernia and lumbar stenosis, but poor results in epidural fibrosis. VEGF is an important factor in the development of angiogenesis (47, 48). It is a multifunctional growth factor with specific effects on endothelial cells in particular (49). The VEGF family has been shown to consist of six members: VEGF-A (Human-VEGF), VEGF-B, VEGF-C, VEGF-D, VEGF-E and Placental growth factor, PlGF (22). VEGF-A has six known isoforms; namely, VEGF121, VEGF145, VEGF165, VEGF183, VEGF189 and VEGF206; the numbers indicating the number of amino acids (50). VEGF is important and necessary for vasculogenesis and angiogenesis, both during development and in adults (51). This growth factor plays a critical role in the development of vessels in particular, but is also necessary for many endothelial cell functions. These physiological and pathophysiological events include embryogenesis, wound healing, tumour growth, myocardial ischemia, ocular neovascular diseases, and inflammatory disorders. Pegaptanib sodium has been proven to be clinically effective by preventing angiogenesis with its anti-VEGF characteristics in neovascular age-related macular degeneration in literature (15, 19, 45, 52). The effect of bevacizumab, which has a mechanism of action similar to pegaptanib sodium, in preventing spinal epidural fibrosis has been demonstrated with the experimental study conducted by Karatay et al (53). We found that the epidural fibrosis in the control group subjects filled the epidural space by completely covering the dura, progressed beneath the pedicle and extended to the spinal roots. However, the fibrosis tissue on the dura was thinner in rats, which received pegaptanib sodium. Epidural fibrosis was intense in the control group but was less prominent in the pegaptanib sodium group, and the difference was statistically significant (p=0,001). Our study was the first study investigating the effect of pegaptanib sodium on spinal epidural fibrosis. We found that topically applied pegaptanib sodium significantly decreased spinal epidural fibrosis with an anti-VEGF effect in rats that had undergone laminectomy.

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