Histopathologic Analysis of Tamoxifen on Epidural Fibrosis

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■ BACKGROUND: **Epidural fibrosis is a challenging topic in spinal surgery. This phenomenon constitutes one of the main reasons behind postlaminectomy syndrome or failed back surgery syndrome, which leads to persistent back and leg pain in association with compression and/or stretching the nerve root or the dura. The exact mechanism of action in epidural fibrosis is complex and remains uncertain. Excessive deposition of collagen, fibronectin, and dermatan sulfate, known as the "extracellular matrix," and decrease of tissue cellularity results in epidural fibrosis. The most investigated and important actor in epidural fibrosis as well as in other forms of aberrant wound healing is presumed to be transforming growth factor-1β formation. Tamoxifen (TAM), a synthetic nonsteroidal antiestrogen used in breast cancer, is also effective in inhibiting fibroblast proliferation via downregulation of transforming growth factor-1β.**

■ METHODS: **Twenty-four adult male rats were randomly divided into 3 groups. Laminectomy was the sole intervention in the control group. Spongostan was placed in the operation lodge after laminectomy in the second group. In the treatment group, TAM was administrated orally after laminectomy. Epidural fibrosis, dural thickness, inflammatory response, and arachnoidal involvement were evaluated and graded histopathologically.**

■ RESULTS: **Epidural fibrosis, dural thickness, and inflammatory response in the subjects treated with TAM were significantly less than in the control and Spongostan group and the differences were statistically significant. Although arachnoidal involvement was observed in a subject in the TAM group, the differences between all groups weren't statistically significant.**

■ CONCLUSIONS: **Tamoxifen reduced epidural fibrosis, dural thickness, and inflammatory response after laminectomy in rats.**

INTRODUCTION

Epidural fibrosis (EF), first defined by LaRocca and Macnab[1] as the laminectomy membrane, is a challenging topic in spinal surgery. Numerous clinical and experimental studies have focused on this issue to clarify and find a way to reduce unsatisfactory results after spinal surgery as it constitutes a major portion of failed back surgery syndrome (FBSS).[2] A systematic review analyzing the results of 6 studies with 663 patients revealed fibrosis to be the third most common cause of FBSS, with an average incidence of 8% after stenosis and disc herniation.[3]

Although EF is the third most common cause of FBSS, one should analyze its contribution to symptoms created by stenosis and disc herniation, where a mild stenosis or a simple bulging would not usually cause any symptoms but the presence of EF only helps to worsen the clinical findings. Even with the rapid improvement of technology and sophisticated surgical strategies in the management of spinal disorders, EF still remains a conundrum. FBSS has an incidence of 4%–50%, with varying

Key words
- Arachnoid
- Epidural fibrosis
- Inflammation
- Laminectomy
- Tamoxifen
- TGF-1β

Abbreviations and Acronyms
EF: Epidural fibrosis
FBSS: Failed back surgery syndrome
TAM: Tamoxifen
TGF-1β: Transforming growth factor-1β

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Table 1. Distribution of Groups

Group	Name	*n*	Process
1	Control	8	Laminectomy
2	Spongostan	8	Laminectomy + Spongostan
3	Tam	8	Laminectomy + tamoxifen

symptoms.[4] Being one of the major constituents of FBSS, EF causes radicular symptoms due to dural tethering.[5] Revision surgery in these cases has greater complication rates along with decreased success rate to 30% after the second, 15% after the third, and 5% after the fourth surgery.[3]

Although the pathophysiology of EF remains complex and uncertain, excessive deposits of fibronectin, dermatan sulfate, and mainly collagen are blamed for decreased cellularity. EF is a multistep cascade eventually leading to inflammation and fibrosis. As in all inflammatory processes, several cytokines and growth factors are released, which in the end are blamed for the progression of fibrosis. One of the most investigated actors of this process is transforming growth factor-1β (TGF-1β), as it is this study's main topic as well.[6]

Numerous agents have been used and their effects on EF have been analyzed, including antiepileptics, nonsteroidal anti-inflammatory drugs, antidepressants, opioids, and interventions such as epidural steroid injections, adhesiolysis, radiofrequency ablation, and neuromodulation.[7] However, only gabapentin, caudal epidural steroid injection, and adhesiolysis proved to have grade I level of evidence in treating EF.[8] Experimental studies focusing on the effect of methylprednisolone, dexamethasone, promethazine, human fibrinolysin, mitomycin C, and numerous agents on EF are present. To reduce or prevent the migration of fibroblasts into the epidural space, many different agents that act as a barrier, such as fat grafts, absorbable gelatin sponges, cellulose mesh, and hyaluronic acid, also have been analyzed.[9] Thus, in this paper, to compare the effect of systematic treatment versus epidural space barrier employment, Spongostan—a hemostatic gelatin sponge—was used.

Tamoxifen (Tam), a well-known synthetic nonsteroidal antiestrogen used primarily in the treatment of breast cancer. has been investigated for its effect on fibrotic diseases via downregulation of TGF-1β and thus inhibiting fibroblast proliferation. Keloid formation,[10] perivascular fibrosis,[11] pulmonary,[12] and renal[13] fibrosis are examples of studies in which Tam's antifibrotic effects have been evaluated. After its discovery in 1972, Tam has not been only used to save lives of patients with breast cancer, but it, along with its derivatives, has been safely used to treat infertility, gynecomastia, retroperitoneal fibrosis, and idiopathic sclerosing mesenteritis along with exerting beneficial effects on osteoporosis and reducing the incidence of cardiovascular diseases. It has also been evaluated for the treatment of Riedel's thyroiditis, bipolar disorder, and McCune-Albright syndrome, with minimal side effects.[14] To date, no known studies focusing on Tam's effect on EF have been published. Taking into consideration that Tam is safely used to treat various disorders besides breast cancer, this study aimed to investigate Tam's effect on EF after laminectomy followed by systemic administration in an experimental rat model.

Table 2. Epidural Fibrosis Grading[15]

Grade 0	Dura mater is free of scar tissue
Grade 1	Thin fibrous bands between scar tissue and dura mater
Grade 2	Continuous adherence in less than two thirds of the laminectomy defect
Grade 3	Widespread adherence of scar tissue affecting more than two thirds of the laminectomy defect and/or adherence to nerve roots

Table 3. Scar Tissue Consistency and Inflammatory Response Grading Table[17]

Grade 0	Loose connective tissue with small collagen bundles, the presence of highly vascular adipose tissue, with moderate macrophage and inflammatory cell activity
Grade 1	Connective tissue density, edges of defect with evidence of new bone formation, and mild macrophage and inflammatory cell activity.
Grade 2	Dense connective tissue and/or fibrocartilage, absence of adipose tissue, avascular tissue, and absence of macrophage 2and inflammatory cell activity
Grade 3	Dense collagenous connective tissue, absence of adipose tissue, avascular tissue, and absence of macrophage and inflammatory cell activity

MATERIALS AND METHODS

The European Communities Council Directive of November 24, 1986 (86/609/EEC) related to the protection of animals for experimental use was strictly followed during the procedures and follow-up animal care. All of the experimental procedures used in this investigation were reviewed and approved by the ethical committee of the Ministry of Health Ankara Research and Training Hospital. A veterinary physician was present throughout the procedures.

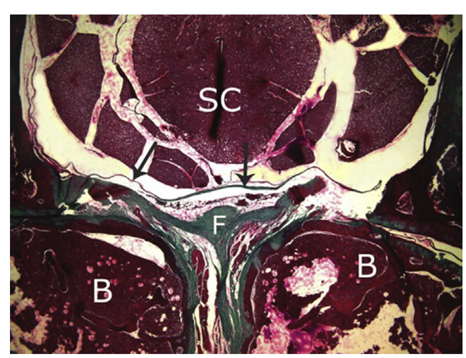

Figure 1. Grade 1 Fibrosis A section from the Tam receiving group showing a thin fibrous band (*arrows*), between the free dura mater and fibrosis (F). SC, spinal cord. Masson's trichrome ×100.

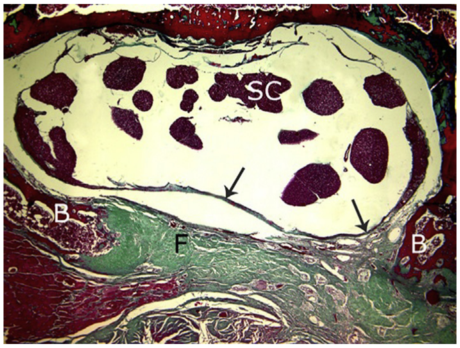

Figure 2. Grade 2 fibrosis histologic examination showing an example of fibrosis (F) covering less than two-thirds of the laminectomy defect and adherence to dura mater (*arrow*). SC, spinal cord. B indicates new bone formation. Masson's trichrome ×100.

Twenty-four adult male Wistar albino rats were used in this experiment. Weighing on average between 250 and 350 g, they were randomly divided into 3 equal groups, as seen in **Table 1**. Laminectomy, as described to follow, was the sole procedure in group 1, and no additional interventions were performed. Group 2 underwent laminectomy as well but a 2 cc/kg saline-soaked Spongostan (Ethicon; Ethicon Endo-Surgery, Inc., Cincinnati, Ohio, USA) was placed on the epidural lodge after the procedure. The same laminectomy procedure was performed for group 3 but the subjects received 40 mg/kg/d Tam (Teva Pharmaceutical Industries Ltd., Petach Tikva, Israel) for 4 weeks.

The rats were kept in separate cages at an average temperature of 22–25°C. Appropriate humidity and a 12-hour light cycle were ensured. The subjects had free access to bait and water. Intraperitoneal xylazine (Rompun; Bayer, Istanbul, Turkey) 10 mg/kg and ketamine hydrochloride (Ketalar; Parke Davis, Istanbul,

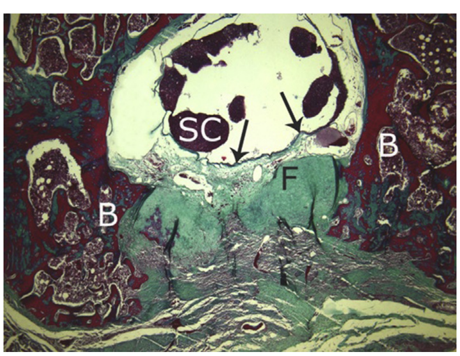

Figure 3. Grade 3 fibrosis A section from the control group. Epidural fibrosis covers more than two-thirds of the laminectomy defect with a wide adherence to dura mater (*arrow*). SC, spinal cord. B indicates new bone formation. Masson's tichrome ×100.

Table 4. Epidural Fibrosis Grades

Groups	Grade 1	Grade 2	Grade 3
Group 1: control	—	2	6
Group 2: Spongostan	3	3	2
Group 3: tamoxifen	5	2	1

Turkey) 50 mg/kg were used for general anesthesia while we closely observed respiration. The rats were then fixated on a board in prone position, and povidone–iodine was used after we shaved the lower backs of the rats to obtain antisepsis.

With a midline incision at about the L3–L5 levels, lumbosacral fascia was observed and incised. Paravertebral muscles were dissected subperiosteally, and the L3–L5 laminae were observed before a total laminectomy along with the excision of ligamentum flavum and epidural adipose tissue. Careful attention was given to observe and not damage the dura mater. Cotton pads were used for hemostasis. Spongostan was placed on the operation lodge in group 2, and all the rats were sutured with 4-0 polypropylene sutures (Ethicon Endo-Surgery, Inc., Cincinnati, Ohio, USA) in their normal anatomic plan. All the procedures were performed by the same surgeon (M.E.Y.) using a surgical microscope (Zeiss OPMI 1; Carl Zeiss Meditec, Oberkochen, Germany). No complications, wound infections, or adverse effects were observed related to the procedure.

Histopathologic Assessment

The subjects were sacrificed 4 weeks after the operation via a lethal injection of pentobarbital (200 mg/kg) (Nembutal; Oak Pharmaceuticals, Lake Forest, Illinois, USA). The lumbar region of the rats was removed en bloc, including the paravertebral muscles, and fixed in 10% buffered formalin for 7 days followed by decalcification for 5 days in ethylenediaminetetraacetic acid/hydrochloric acid solution. The site of laminectomy was identified, and 2-mm sections were obtained, which were then embedded in paraffin. A microtome was used to obtain serial sections of 5-μm slides then stained with hematoxylin & eosin and Masson's trichrome stains.

A pathologist blinded to the study evaluated the sections for dural thickness and EF under a light microscope (Eclipse 80i; Nikon Instruments, Inc., Melville, New York, USA). The Nikon Nis Elements D 3.1 Digital Analyzing System was used to perform quantitative morphometric analysis. Mean values were taken into consideration for statistical evaluation.

In physiological spinal cord anatomy, a thin arachnoid membrane is present that is free from the dura mater. However, the arachnoid membrane can be thickened and adhered to dura mater in pathologic conditions defined as arachnoidal involvement. Thus, this parameter also was evaluated. Fibroblast and inflammatory cell count was examined to evaluate the extent of the inflammatory process.

The tissue samples were fixed in phosphate-buffered 10% formalin for 7 days followed by tap water clearance and decalcification in 25% formic acid for 5 days. The samples were washed

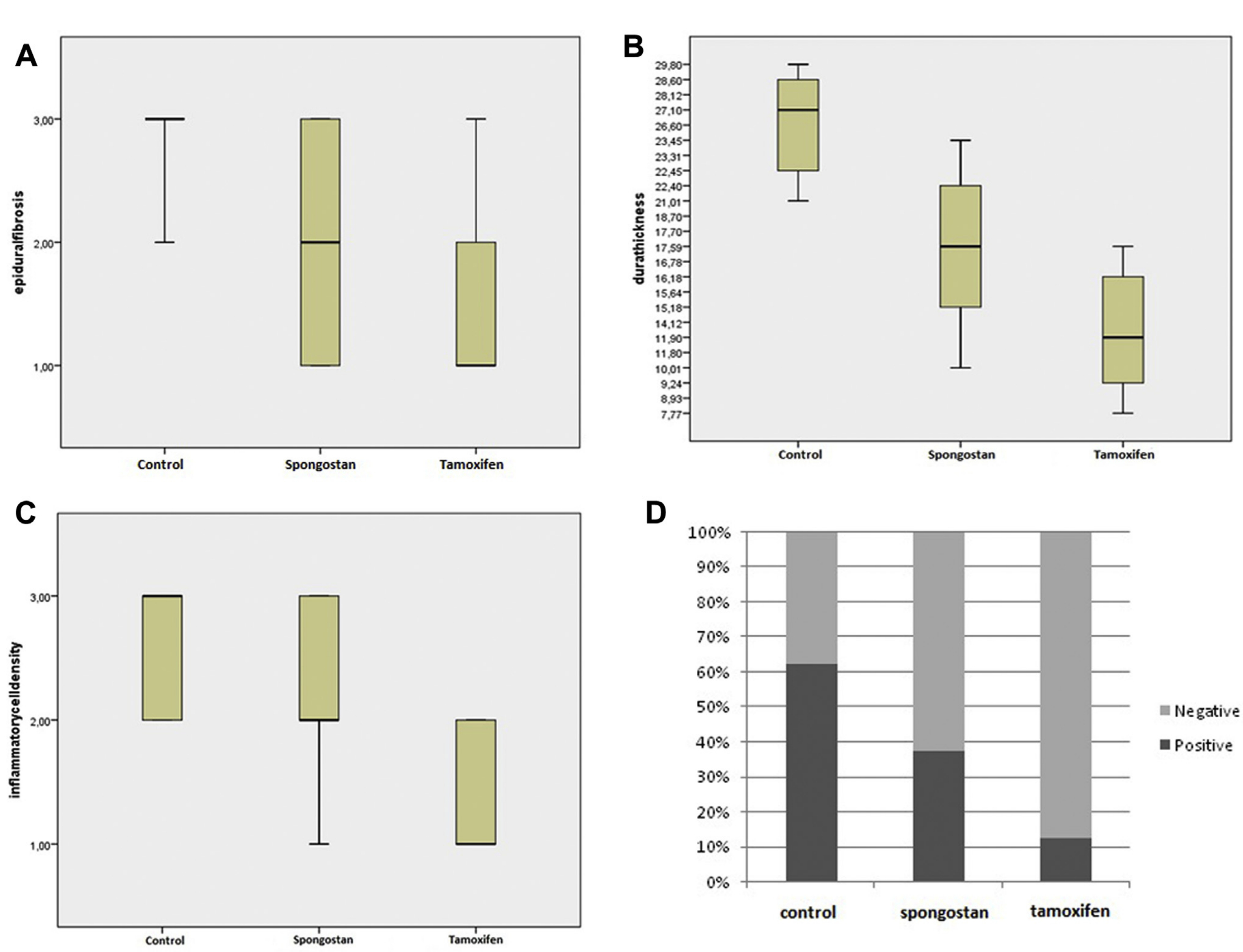

Figure 4. (**A**) Comparison of epidural fibrosis grades among groups. The *horizontal lines* in the middle of each box indicate the median, whereas the *top* and *bottom borders* of the box mark the 25th and 75th percentiles, respectively. The *whiskers* above and below the box mark the maximum and minimum epidural fibrosis grades. (**B**) Comparison of dural thickness among groups. The *horizontal lines* in the middle of each box indicate the median, whereas the *top* and *bottom borders* of the box mark the 25th and 75th percentiles, respectively. The *whiskers* above and below the box mark the maximum and minimum dural thickness. (**C**) Comparison of inflammatory cell density grades among groups. The *horizontal lines* in the middle of each box indicate the median, whereas the *top* and *bottom* borders of the box mark the 25th and 75th percentiles, respectively. The *whiskers* above and below the box mark the maximum and minimum inflammatory cell density grades. *Asterisks* represent extreme cases. (**D**) Comparison of arachnoidal involvement among groups. The *light gray bars* indicate the ratio of arachnoidal involvement negative within each group; the prevalence of arachnoidal involvement positive is shown as *dark gray bars*.

in tap water followed by dehydration in a graded ethanol series, cleared in xylene, and embedded in paraffin blocks. Then, 5-µm sections were obtained with a microtome (Leica RM2125 RTS; Leica Biosystems, Inc., Buffalo Grove, Illinois, USA) and stained with hematoxylin and eosin and Masson's trichrome stain.

Slides were examined and photographed with the Axio Scope-A1 (Carl-Zeiss, Oberkochen, Germany) microscope at 100× magnification. All sections were evaluated by the same pathologist, who was blinded to the study, for EF, dura thickness, arachnoidal involvement, scar tissue consistency, and inflammatory response.

EF was graded according to He et al.[15] (Table 2). Dura thickness was measured via the Axiovision software program (Carl-Zeiss) at the 3 predetermined segments described by Cemil et al.[16]; the first measurement was taken from the midpoint of the laminectomy defect, the second 2 mm right of the first sample, and the third 2 mm left of the first sample.

To evaluate the scar tissue consistency and inflammatory response, the scoring system proposed by Miyamoto et al.[17] was used based on a 4-point scale (Table 3). Arachnoidal adhesions to the dura mater along with increased thickness also were noted.

STATISTICAL ANALYSIS

Data analysis was performed via SPSS for Windows, version 11.5 (SPSS, Inc., Chicago, Illinois, USA). The Shapiro–Wilk test was used to determine whether the distributions of continuous

Table 5. Dural Thickness Measured at Three Different Points

	1.rat	2.rat	3.rat	4.rat	5.rat	6.rat	7.rat	8.rat	Overall Mean
Group 1: control	25.7	23.7	35.29	30.14	26.2	20.43	28.14	17.3	24.71
	27.1	26.6	21.01	28.12	23.31	28.6	29.8	22.4	
	23.57	16.23	19.32	34.5	19.37	19.4	30.82	16.1	
Group 2: Spongostan	8.27	15.78	21.08	20.98	24.08	8.2	24.16	9.2	17.45
	10.01	18.7	22.45	17.7	23.45	14.12	16.78	15.18	
	16.5	23.09	20.7	25.09	21.37	16.8	14.72	10.52	
Group 3: tamoxifen	8.54	19.19	14.59	18.12	8.64	18.24	7.58	9.62	13.46
	7.77	17.59	15.64	11.8	9.24	16.18	8.93	11.9	
	9.20	19.99	21.52	16.12	13.14	18.52	10.32	10.83	

Measurements are in micrometers (μm).

variables were normal. The nonparametric Kruskal—Wallis test was used to compare differences in groups. P values used to determine whether the differences were statistically significant. A P value less than 0.05 was considered statistically significant.

RESULTS

No mortality or morbidity related to the procedure occurred. All animals were ambulatory at the time of euthanasia. There was no wound infection, erythema, or cerebrospinal fluid leakage observed during the study. Various grades of fibrosis observed under Masson's trichrome stain are given below (Figures 1—3).

As seen in Table 4, the control group had no grade 1 EF and consisted majorly of grade 3 EF with 75%. Group 2 had a more even distribution with 37.5%, 37.5%, and 25% grades, respectively. In the Tam-receiving group 3, EF grades were as follows; grade 1: 62.5%, grade 2: 25%, and grade 3: 12.5%. The difference between the control and the Spongostan group ($P = 0.03$) and the difference between the control and the Tam group were both statistically significant ($P = 0.005$) (Figure 4A). However, the difference between Spongostan and Tam group was not statistically significant ($P = 0.330$).

Table 5 shows the measurements of dura mater thickness as described by Cemil et al.[16] in micrometers. The mean thickness of dura was 0.024 mm in the control group, 0.017 mm in the Spongostan group, and 0.013 mm in the Tam group. The differences between the Tam group compared with the control and Spongostan groups were statistically significant ($P = 0.001$, $P = 0.036$ respectively). The difference between the control and Spongostan group was also statistically significant ($P = 0.005$) (Figure 4B).

Table 6 summarizes effects on inflammatory cell density. The control group had an equal grade 2 and 3 distribution with 4 in each grade. The Spongostan group had a distribution of 25%, 50%, and 25%, respectively, whereas most of the subjects in the Tam group were in grade 1 with 75% and grade 2 with 25%. When the Tam group was compared with the control and the Spongostan group, a statistically significant result was found for both comparisons ($P = 0.002$, $P = 0.037$, respectively). The difference between the control and Spongostan group did not yield a statistically significant result ($P = 0.165$) (Figure 4C).

Arachnoidal involvement was observed in a single subject in the Tam group (12.5%). Although the control group had a 62.5% and the Spongostan group 37.5% positive arachnoidal involvement (Table 7). The differences between all groups did not yield a statistically significant result ($P = 0.129$, Kruskal—Wallis Test) (Figure 4D). All comparisons are summarized in Table 8.

DISCUSSION

FBSS continues to be a challenge even with the advancements of surgical techniques and treatment modalities. Although the worldwide surgical trend is pushing toward a more minimally invasive approach, a 170.9% increase in lumbar fusion surgery and 11.3% increase in laminectomies have been observed between the years of 1998 and 2008,[7] leading to an increased number of EF, an

Table 6. Scar Tissue Consistency and Inflammatory Response Grade Results

Groups	Grade 1	Grade 2	Grade 3
1: control	—	4	4
2: Spongostan	2	4	2
3: tamoxifen	6	2	—

Table 7. Arachnoidal Involvement Results

Groups	(+)	(−)
1: control	5	3
2: Spongostan	3	5
3: tamoxifen	1	7

Table 8. Comparisons of Variables Measured in the Study

Variables	Control	Spongostan	Tamoxifen	*P* Value*
Epidural fibrosis	3 (2–3)†,‡	2 (1–3)‡	1 (1–2)†	0.011
Dural thickness	27.10 (21.01–29.80)†,‡	17.59 (10.01–23.45)‡,§	11.9 (7.77–17.59)†,§	<0.001
Inflammatory cell density	3 (2–3)†	2 (1–3)§	1 (1–2)†,§	0.005
Arachnoid involvement	5/3	3/5	1/7	0.129

Data were represented as median (25–75 percentile).
*Kruskal–Wallis test.
†Tamoxifen group versus the control group ($P < 0.01$).
‡Spongostan group versus the control group ($P < 0.05$).
§Tamoxifen group versus the Spongostan group ($P < 0.05$).

expected healing consequence after laminectomy. A major constituent of FBSS is EF, otherwise known as postlaminectomy membrane.[5] Several studies support EF to be one of the top 3 causes of FBSS.[3,18] In addition, the first 2 causes of FBSS are stenosis and disc herniation. However, a plausible argument in these primary 2 causes would be that EF aggravates the clinical symptoms caused by stenosis or disc herniation where a previous intervention has been performed. Thus, if patients included in the first 2 causes with EF were dismissed, EF may become the leading cause of FBSS.

Although the pathophysiologic mechanism of excess fibrosis formation has not been elucidated, the role of inflammation in wound healing as the first step of scar formation has been blamed. The migration of lymphocytes, fibroblasts, and macrophages into the epidural space start the cascade for scar formation, which mainly consists of fibronectin and collagen fibers. Formation of EF requires the transformation of fibroblasts into myofibroblasts, which is mediated by TGF-1β,[19] leading to the purpose of this study—the pathophysiology underlying EF may be driven by TGF-1β.

A selective estrogen receptor modulator, Tam has been widely used for breast cancer treatment and prevention for about half a century. In vitro studies along with numerous clinical studies of desmoid tumors and retroperitoneal fibrosis treated with Tam have been reported. Tam's antifibrotic effect acts via altered RNA transcription, decreased cellular proliferation, delay or arrest of the cell cycle, and inhibition of growth factors such as TGF and insulin-like growth factor,[20] leading to the conclusion that Tam has antifibrotic effects on connective tissue.

Clinical studies have shown Tam to reduce pulmonary fibrosis and intra-abdominal adhesion via reducing the production of TGF-β and insulin-like growth factor-1. TGF-β is known to play a key role in the modulation of inflammation and scar formation by suppressing lymphocyte proliferation and anchorage-independent growth of fibroblasts.[21,22]

Additional uses of Tam have been proven to be successful in the treatment of fibrosclerotic disorders, including idiopathic retroperitoneal fibrosis, encapsulating peritoneal fibrosis, fibrosing mediastinitis, and desmoid tumors. Researchers have concluded that Tam decreases dermal fibroblast proliferation along with excessive extracellular matrix production in mesangial cells.[23]

Kim et al.[23] evaluated the protective effect of Tam on renal fibrosis induced by unilateral urethral obstruction and revealed that Tam decreased unilateral urethral obstruction–induced renal tubular injury, interstitial matrix deposition, and inflammatory processes. They also concluded that Tam inhibited TGF-β1–induced renal fibroblast activation through suppression of ERα-mediated TGF-β1/Smad signaling pathway.[23]

Another study focusing on Tam as a monotherapy treatment of retroperitoneal fibrosis concluded that it is a promising alternative approach through its anti-inflammatory and antifibroblastic activity. These effects allowed Tam to be used in the treatment of Peyronie's disease and pelvic fibromatosis as well. Tam administration was tolerated well by patients, and only 22.6% of patients were taken off medication due to side effects after a mean duration of 5.1 months. The most common mild side effect was malaise.[24] This selective study on human patients would support the short-term administration of Tam to prevent EF. In vitro studies based on fibroblast-populated collagen lattice demonstrated that Tam inhibited normal fibroblast function contraction along with keloid fibroblasts, Dupuytren's affected palmar fascia fibroblasts, and fibroblasts derived from rhinophymas.[25]

In light of all these studies, it could be concluded that Tam reduces TGF-β and synthesis of collagen in mesangial cell culture, inhibits human dermal fibroblast proliferation, decreases fibroblast function, and inhibits wound contraction.[13,19] With this knowledge, numerous clinical studies have evaluated Tam's effect in the treatment of sclerotic disorders, but no study focusing on its effect on EF has been published.

Various chemotherapeutic agents have been used for their antifibrotic effects on EF, such as methotrexate,[26] bevacizumab,[27] temozolomide,[28] mitomycin C,[29] 5-fluorouracil, and cyclosporin A,[30] which all proved beneficial. The notion that anticancer drugs may be used to inhibit fibroblast proliferation in the epidural space to prevent fibrosis is one of the leading causes of this study.

Methotrexate, a folic acid antagonist used as a chemotherapy agent and immune system suppressant, has been previously evaluated in a similar study[26] involving 48 rats in whom laminectomy was performed yielded promising results similar to this study. However, in a study comparing the effect of methotrexate versus Tam on abortion rates, Tam was found to have fewer side effects in the first phase.[31] A chemotherapeutic agent does not come without side effects, and in a different study evaluating the benefits and cost effectiveness,[32] Tam was found to be cost effective in the treatment of breast cancer but did raise issues regarding increased risk of endometrial cancer and pulmonary embolism. Thus, pre-existing risks for these conditions must be evaluated and monitored during the treatment period to safely use Tam for the treatment of EF. Nonetheless, these serious increased risks were observed in 5-year follow-up periods. Chemotherapeutic agents mostly inhibit growth factors and cytokines released primarily in the acute phases of wound healing. The subjects of this study were euthanized 4 weeks after the surgery, providing enough time for a healthy rat to complete the wound healing process. Hence, observing the side effects caused by Tam in a short-term treatment would allow for a healthier decision for the treatment period.

Bevacizumab, an antivascular endothelial growth factor agent used primarily in the treatment of colorectal cancer, has also proven to be beneficial in reducing EF[27] by altering angiogenesis. However, serious cardiovascular adverse effects were observed as it inhibits vascular endothelial growth factor, leading to decreased nitric oxide, which in the end promotes vasoconstriction resulting in bevacizumab-induced arterial hypertension at a risk rate of 3- to 7.5-fold.[33] Temozolomide, another agent used to reduce EF, has a high incidence of hematologic side effects such as aplastic anemia and myelodysplastic syndrome.[34] It must be kept in mind, however, that these side effects of chemotherapeutic agents are evaluated during the treatment of a malignancy, thus requiring a longer term of treatment. Inhibiting fibrosis causing cytokines in normal wound healing would only require treatment for the initial phases lasting until the remodeling phase, which usually starts 3 days after the trauma.[35]

To compare the cost effectiveness of Tam versus other chemotherapeutic agents experimented on EF; the average price of the most prescribed brand of each generic agent is given in Table 9. In short, Tam is less expensive when compared with other agents.

It was this paper's aim to examine Tam's effectiveness in inhibiting fibroblast proliferation for a challenging problem to all spinal surgeons; EF. EF is one of the leading causes of FBSS and can create symptoms similar to or worse than the initial presenting symptoms. Most spinal surgeries consist of laminectomy, that is, removing the lamina and ligamentum flavum allowing the epidural space to be visualized clearly. Postoperatively, however, this potential space allows for scar tissue formation, which may cause radiculopathic symptoms via dura thickening, dural adhesions, and compression of nerve roots.[36] Spinal surgeons must be aware of the importance of preserving ligamentum flavum during laminectomy or laminotomy. A comprehensive study involving 400 patients revealed that preserving the ligamentum flavum reduced postoperative fibrosis in a follow-up period of 4 weeks, along with a decreased number of symptoms and reoperation rates.[37] Postoperative agents may aid the process of preventing EF and may be used in the near future, but this does not shadow the importance of microsurgical techniques and maximal preservation of healthy tissues.

Table 9. Prices of Chemotherapeutic Agents Used Previously for Epidural Fibrosis

Generic Name	Brand Name	Price (USD)
Tamoxifen	Nolvadex	$6.77
Bevacizumab	Altuzan-Roche	$819.62
Temozolomide	Temodal	$79.81
Mitomycin C	Mitomycin-C Kyowa	$51.79
5-Fluorouracil	Fluorouracil-Farmako	$20.23
Cyclosporin	Epostin	$58.71

Prices were obtained from RxMediaPharma 2017, a nationwide prescription follow-up program in Turkey, given as U.S. dollars. Accessed December 26th, 2017.

According to this study's results, EF in Tam group was lower compared with the control group ($P = 0.005$). Dural thickness was lower in the Tam group compared with the control and Spongostan group ($P = 0.001$, $P = 0.036$, respectively). Also, Tam proved to be effective in reducing inflammatory response compared with the control and Spongostan groups ($P = 0.02$, $P = 0.037$, respectively). Although no statistically significant result was obtained when arachnoidal involvement was compared, it is to be noted that only a single rat presented with arachnoidal involvement in the Tam group.

This preliminary experimental study on rats after laminectomy supports the conclusion that Tam may be a safe and effective treatment modality in reducing EF, inflammation, and dural thickness. Although a myriad of mechanisms have been suggested to clarify the cascade of EF, as reported in the relevant literature the administration of Tam may inhibit TGF-1β and related pathways, which is crucial in the inflammation and scar tissue process.

CONCLUSIONS

Numerous agents have been evaluated for their effect on EF. Tam, a well-known selective estrogen receptor modulator, also has been evaluated for its antifibrotic effects. This paper is the first study focusing on Tam's effect on EF and concludes that it proved to be beneficial in reducing EF, although the exact mechanism, dosage, and duration investigation needs to be further evaluated.

REFERENCES

1. LaRocca H, Macnab I. The laminectomy membrane. Studies in its evolution, characteristics, effects and prophylaxis in dogs. *J Bone Joint Surg Br*. 1974;56B:545-550.
2. Jinkins JR, Osborn AG, Garrett D Jr, Hunt S, Story JL. Spinal nerve enhancement with Gd-DTPA: MR correlation with the postoperative lumbosacral spine. *AJNR Am J Neuroradiol*. 1993;14:383-394.
3. Clancy C, Quinn A, Wilson F. The aetiologies of failed back surgery syndrome: a systematic review. *J Back Musculoskelet Rehabil*. 2017;30:395-402.
4. Taylor RS, Taylor RJ. The economic impact of failed back surgery syndrome. *Br J Pain*. 2012;6:174-181.
5. Mohi Eldin MM, Abdel Razek NM. Epidural fibrosis after lumbar disc surgery: prevention and outcome evaluation. *Asian Spine J*. 2015;9:370-385.
6. Zhu J, Li Y, Shen W, Qiao C, Ambrosio F, Lavasani M, et al. Relationships between transforming growth factor-beta1, myostatin, and decorin: implications for skeletal muscle fibrosis. *J Biol Chem*. 2007;282:25852-25863.

7. Baber Z, Erdek MA. Failed back surgery syndrome: current perspectives. *J Pain Res*. 2016;9:979-987. eCollection 2016.

8. Amirdelfan K, Webster L, Poree L, Sukul V, McRoberts P. Treatment options for failed back surgery syndrome patients with refractory chronic pain: an evidence based approach. *Spine (Phila Pa 1976)*. 2017;42(suppl 14):S41-S52.

9. 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*. 2010;10:441-447.

10. Soares-Lopes LR, Soares-Lopes IM, Filho LL, Alencar AP, da Silva BB. Morphological and morphometric analysis of the effects of intralesional tamoxifen on keloids. *Exp Biol Med (Maywood)*. 2017;242:926-929.

11. Wermuth PJ, Carney KR, Mendoza FA, Piera-Velazquez S, Jimenez SA. Endothelial cell-specific activation of transforming growth factor-β signaling in mice induces cutaneous, visceral, and microvascular fibrosis. *Lab Invest*. 2017;97:806-818.

12. Koc M, Polat P, Suma S. Effects of tamoxifen on pulmonary fibrosis after cobalt-60 radiotherapy in breast cancer patients. *Radiother Oncol*. 2002;64:171-175.

13. Dellê H, Rocha JRC, Cavaglieri RC, Vieira JM, Malheiros DMAC, Noronha IL. Antifibrotic effect of tamoxifen in a model of progressive renal disease. *J Am Soc Nephrol*. 2012;23:37-48.

14. Shagufta, Ahmad I. Tamoxifen a pioneering drug: an update on the therapeutic potential of tamoxifen derivatives. *Eur J Med Chem*. 2018;143:515-531.

15. He Y, Revel M, Loty B. A quantitative model of postlaminectomy scar formation. Effects of a nonsteroidal antiinflammatory drug. *Spine*. 1995;20:557-563.

16. Cemil B, Tun K, Kaptanoglu E, Kaymaz F, Cevirgen B, Comert A, et al. Use of pimecrolimus to prevent epidural fibrosis in a postlaminectomy rat model. *J Neurosurg Spine*. 2009;11:758-763.

17. Miyamoto K, Masuda K, Inoue N, Okuma M, Muehleman C, An HS. Anti-adhesion properties of a thrombin-based hemostatic gelatin in a canine laminectomy model: a biomechanical, biochemical, and histologic study. *Spine (Phila Pa 1976)*. 2006;31:E91.

18. Slipman CW, Shin CH, Patel RK, Isaac Z, Huston CW, Lipetz JS, et al. Etiologies of failed back surgery syndrome. *Pain Med*. 2002;3:200-214 [discussion: 214-217].

19. Chau D, Mancoll JS, Lee S, Zhao J, Phillips LG, Gittes GK, et al. Tamoxifen downregulates TGF-beta production in keloid fibroblasts. *Ann Plast Surg*. 1998;40:490-493.

20. Hu D, Hughes MA, Cherry GW. Topical tamoxifen—a potential therapeutic regime in treating excessive dermal scarring? *Br J Plast Surg*. 1998;51:462-469.

21. Karaca T, Gözalan AU, Yoldaş Ö, Bilgin BÇ, Tezer A. Effects of tamoxifen citrate on postoperative intra-abdominal adhesion in a rat model. *Int J Surg*. 2013;11:68-72.

22. Karaca T, Yoldas O, Bilgin BC, Yilmaz OH, Karaca N, Simsek GG, et al. Does tamoxifen citrate prevent pulmonary fibrosis due to silica inhalation? *Bratisl Lek Listy*. 2015;116:440-445.

23. Kim D, Lee AS, Jung YJ, Yang KH, Lee S, Park SK, et al. Tamoxifen ameliorates renal tubulointerstitial fibrosis by modulation of estrogen receptor α-mediated transforming growth factor-β1/Smad signaling pathway. *Nephrol Dial Transpl*. 2014;29:2043-2053.

24. Brandt AS, Kamper L, Kukuk S, Haage P, Roth S. Tamoxifen monotherapy in the treatment of retroperitoneal fibrosis. *Urol Int*. 2014;93:320-325.

25. Ryu SH, Chung YH, Lee JK, Kim JA, Shin JW, Jang MK, et al. Antifi brogenic effects of tamoxifen in a rat model of periportal hepatic fibrosis. *Liver Int*. 2009;29:308-314.

26. Chen H, Yan L, Wang J, Sun Y, Li X, Zhao S, et al. Methotrexate prevents epidural fibrosis through endoplasmic reticulum stress signalling pathway. *Eur J Pharmacol*. 2017;796:131-138.

27. 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*. 2012;22:753-757.

28. Aydincak O, Yilmaz MB, Emmez H, Kurt G, Sepici A, Memis L, et al. The effect of temozolomide on the prevention of epidural fibrosis developing after lumbar laminectomy in rats. *Turk Neurosurg*. 2012;22:706-711.

29. Sui T, Ge DW, Yang L, Tang J, Cao XJ, Ge YB. Mitomycin C induces apoptosis in human epidural scar fibroblasts after surgical decompression for spinal cord injury. *Neural Regen Res*. 2017;12:644-653.

30. Yildiz KH, Gezen F, Is M, Cukur S, Dosoglu M, Mitomycin C. 5-fluorouracil, and cyclosporin A prevent epidural fibrosis in an experimental laminectomy model. *Eur Spine J*. 2007;16:1525-1530.

31. Wiebe ER. Tamoxifen compared to methotrexate when used with misoprostol for abortion. *Contraception*. 1999;59:265-270.

32. Eckermann SD, Martin AJ, Stockler MR, Simes RJ. The benefits and costs of tamoxifen for breast cancer prevention. *Aust N Z J Public Health*. 2003;27:34-40.

33. Gherman A, Căinap C, Constantin AM, Cetean S, Căinap SS. Molecular targeted treatment of metastatic colorectal cancer: the cardiovascular adverse effects of Bevacizumab and Cetuximab. *Clujul Med*. 2017;90:377-384.

34. Villano JL, Letarte N, Yu JM, Abdur S, Bressler LR. Hematologic adverse events associated with temozolomide. *Cancer Chemother Pharmacol*. 2012;69:107-113.

35. Broughton G 2nd, Janis JE, Attinger CE. The basic science of wound healing. *Plast Reconstr Surg*. 2006;117(7 suppl):12S-34S.

36. Gurcay AG, Kazanci A, Gurcan O, Bozkurt I, Onder E, Altinel F, et al. Evaluation of topical application of polysaccharide hemostat and oxidized regenerated cellulose on epidural fibrosis in rats. *J Neurol Sci [Turkish]*. 2016;33:278-285.

37. Aydin Y, Ziyal IM, Duman H, Türkmen CS, Başak M, Sahin Y. Clinical and radiological results of lumbar microdiskectomy technique with preserving of ligamentum flavum comparing to the standard microdiskectomy technique. *Surg Neurol*. 2002;57:5-13 [discussion: 13-14].

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