Evaluation of Topical Application of Polysaccharide Hemostat and Oxidized Regenerated Cellulose on Epidural Fibrosis in Rats

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Summary

Introduction: Post laminectomy syndrome caused by epidural fibrosis (EF) is an annoying problem for all spinal surgeons. Even after a successful operation EF may mimic previous symptoms giving rise to failed back surgery syndrome (FBSS). Although adhesive barriers are employed to reduce epidural scarring, whether or not hemostatic agents influence EF is yet not clear.

Objectives: To elucidate the effects of commonly used hemostatic agents in neurosurgery on epidural fibrosis after laminectomy on rats.

Methods: This experimental study involving rats after laminectomy, an agent known to decrease EF ADCON®-L was compared with polysaccharide hemostat (SealFoam®) and oxidized regenerated cellulose (Surgicel®). 32 Sprague-Dawley rats were divided into four groups, all had laminectomies and their epidural space was examined after 45 days.

Results: When compared with the control group, both SealFoam® and Surgicel® had a decreased grade of EF which was statistically significant (p=0.001). Although not statistically significant when compared with ADCON®-L, these agents had similar effects on reducing epidural fibrosis.

Conclusion: Hemostatic agents are commonly used in spine surgeries. Post operative local hematoma increases epidural fibrosis thus pushing surgeons toward using more agents and leaving them behind. This study shows that polysaccharide hemostat and oxidized regenerated cellulose do not enhance but decrease epidural fibrosis following laminectomy in rats, suggesting that it is not necessary to remove these agents from the surgery field after achieving homeostasis during spinal surgery.

Key words: Epidural fibrosis, laminectomy, polysaccharide hemostat, oxidized regenerated cellulose

Polisakkarit Hemostat ve Okside Rejenere Selüloz'ün Şıçanlarda Laminektomi Sonrası Epidural Fibrozis Üzerine Etkisi

Özet

Giriş: Laminektomi sonrası gelişen epidural fibrosis (EF) cerrahlar için önemli bir problemdir. Sorunsuz geçten ameliyattan sonra gelişen EF, ameliyat öncesi kadar şiddetli sempptomlara yol açabilir ve başarısız bel cerrahisi sendromuna (BBCM) neden olabilir. Adezyon önleyici bariyerlerin EF'i azalttığı bilinmektedir ancak cerrahi sırasında kullanılan hemostatik ajanların epidural fibrozis üzerine etkisi yeteri kadar araştırılmamıştır.
Amaç: Bu çalışmada, spinal cerrahi pratikinde kullanılan hemostatik ajanların deneySEL hayvan modelinde EF üzerine etkisi aydınlatılmaya çalışılmıştır.
Yöntem: 32 Sprague-Dawley sıçan 8'erli 4 gruba ayrılrı ve hepsine laminektomi yapılır. Bir gruba hiçbir madde uygulama dan kontrol grubu olarak ayrılırı, 2. gruba polisakkarit hemostat (SealFoam®), 3. gruba okside rejenere selüloz (Surgicel®) ve 4. gruba epidural fibrozisi azalttığı bilinen ve bu amaçla kullanılan Adcon®-L uygulanır. 45 gün sonra sacrifiye edilen sıçanların dokuları histopatolojik olarak değerlendirildi.
Bulgular: Kontrol grubu ile karşılaştırıldığında SealFoam® ve Surgicel® gruplarının her ikisinde istatistiksel olarak anlamlı düzeyde epidural fibrozisi azalttığı tespit edildi (p=0.001). SealFoam® ve Surgicel® grupları Adcon®-L grubu ile karşılaştırıldığında istatistiksel olarak anlamlı fark bulunmadı. Bu sonuç da EF üzerine Surgicel® ve SealFoam®'in Adcon®-L'e benzer etkiye sahip olduğunu göstermiştir.
Sonuç: Spinal cerrahi sonrası lokal hematomun EF'i arttırduğu bilinmektedir ve bu etkiye azaltmak için hemostaz sağlamak önemlidir. Bu çalışmada deneySEL hayvan modelinde laminektomi sonrası uygulanan polisakkarit hemostat ve okside rejenere selüloz hemostatik ajanların EF'i anımsal ölçüde azalttığı gösterilmiştir.

Anahtar Kelimeler: Epidural fibrozis, laminektomi, polisakkarit hemostat, rkside rejenere selüloz

INTRODUCTION

Since the introduction of sciatica and its etiology various treatment methods have been improvised but up to date disc herniation being the most common reason, the most effective treatment is still considered to be surgery. On average a lumbar disc herniation operation is the most frequent procedure performed by spinal surgeons. The surgeon's main goal aside from correcting neurological deficit is to alleviate the patient of the unbearable pain. Thus a failed back surgery syndrome troubles the minds of surgeons. A common reason for this is epidural fibrosis (EF) also known as “post-laminectomy syndrome” and occurs in 24% of patients operated for lumbar disc herniation (LDH). Epidural fibrosis creates a similar clinic to that of LDH causing nerve root or dura mater compression.(8) Also revision surgeries have higher rates of complications mostly due to disrupted anatomical plane such as radix injuries, epidural hemorrhage and dural tear often ending in cerebrospinal fluid leakage.(2)

There is yet no definite technique to reduce EF formation after lumbar surgery. Even minimally invasive techniques create as much EF as classical methods(13). Thus EF will continue to present as a problem until an effective modality arises. Post operative formation of epidural fibrosis is a multi-step process that involves the reduction of tissue cellularity along with increased deposition of extracellular matrix (ECM) components such as collagen, fibronectin and dermatan sulfate(11). Transforming growth factor-beta 1 plays a critical role within this process by initiating and promoting fibrosis through transdifferentiation of fibroblasts to myofibroblasts(19).

ADCON®-L is a bioreserobable carbohydrate polymer gel used routinely during spine surgery to reduce peridural scarring and adhesions specifically for recurrent pain after lumbar disc herniation. While there is still an argument whether ADCON®-L reduces scar formation and adhesions numerous publications prove otherwise(14,10,9). Although a complex multi step in short, epidural fibrosis involves the migration of fibroblasts onto the nerve tissues where normally epidural adipose tissue would allow the nerve structures to move freely without any compression. Fibroblasts eventually form fibrotic tissue
compressing or stretching nervous structures to neighboring tissues\(^{(12)}\). ADCON\(^{®}\)-L is suggested to prevent epidural scarring by covering nervous structures until fibrosis is complete and is degraded in 3-4 weeks. Some case reports have stated increased risk of cerebrospinal fluid leakage however experience in this clinic along with numerous publications report no net increase of complications making it a safe way to reduce scarring\(^{(7)}\).

SealFoam\(^{®}\) absorbable polysaccharide hemostat is a plant based absorbable hemostatic foam and is absorbed in 3 days. It contains no human or animal parts since it is derived from purified plant starch. The hydrophilic feature of the product dehydrates the blood within the surgical site allowing a higher concentration of platelets and coagulation factors. With its adhesive effect it binds to the surgical site to decrease the chances of rebleeding.

Surgicel\(^{®}\) is as an oxidized cellulose preparation, gauzelike, thrombogenic material that is inert and bioabsorbable. The hemostatic action of Surgicel\(^{®}\) is by formation of a gelatinous mass upon saturation with blood, which leads to formation of a stable clot.

Despite the fact spine surgeons rarely employ materials intraoperatively to decrease epidural fibrosis, hemostatic agents are frequently and abundantly used. The producers of hemostatic agents advice of removing excess agent after hemostasis is achieved but most surgeons tend to leave behind the hemostatic agents to avoid taking chances of a rebleed. However, there is not enough evidence up to date about the relationship between hemostatic agents and epidural fibrosis. The purpose of this study was to investigate the effects of topical polysaccharide hemostat (SealFoam\(^{®}\) Starch Medical, USA) and oxidized regenerated cellulose (Surgicel\(^{®}\) Absorbable Hemostat, Ethicon, USA) on epidural space.

### MATERIAL AND METHODS

This experimental study was conducted in the laboratory of Kobay Experimental Animals Incorporated Company, Ankara after obtaining the approval of the ethic committee (19.03.2015/139). 32 adult male Wistar albino rats weighing on average 300-350g were used in this study. A veterinarian was present throughout the process to minimize the discomfort of the animals. They were divided into 4 groups.

- **Group 1**: Control (n=8) Only lumbar laminectomy was performed.
- **Group 2**: ADCON\(^{®}\)-L (n=8) Lumbar laminectomy + ADCON\(^{®}\) was applied.
- **Group 3**: SealFoam\(^{®}\) (n=8) Lumbar laminectomy + SealFoam\(^{®}\) was applied.
- **Group 4**: Surgicel\(^{®}\) (n=8) Lumbar laminectomy + Surgicel\(^{®}\) was applied.

The rats were given 60 mg/ kg of ketamine hydrochloride intraperitoneally (Alfamine 10%, Egevet Veterinary Services) and 5 mg/kg of xylazin (Alfazyne 2%, Egevet Veterinary Services) for general anesthesia. After sedation the rats were placed on the fixation board in prone position, the lumbar region was shaved and cleansed with povidone iodine. A midline incision at T12-L4 level was performed, the paravertebral muscles were split and the laminae were exposed. A total posterior laminectomy was performed with uttermost caution not to traumatize the spinal cord.

After visualizing the dura mater, the control group was sutured without any additional maneuver. The rats in the other group receiveded ADCON\(^{®}\)-L, SealFoam\(^{®}\) and Surgicel\(^{®}\) respectively. The rats were kept in separate cages for 45 days at 35-37\(^\circ\) C. They continued to receive bait and water and were closely monitored. At the end of 45 days the rats were again sedated using the same method, the previous operation site was incised and the spinal cord at the level of laminectomy was removed along with 1 vertebra level above.
and below the surgical site. The rats were then euthanized by an incision at the right atrium.

**Histological Analysis**

Pathological sampling was carried out after formalin fixation, decalcification and rehydration. All tissue layers of vertebral column including the spinal cord, epidural space, bony structures and muscles were sampled enblock. Paraffin embedded tissue blocks were prepared and 2 sections were obtained from each block. Primary microscopical examination was based on the Haematoxylin and eosin (HE) stained sections. Presence or absence of fibrosis was further confirmed by Masson Trichrome (MT) stain. The fibrotic process in epidural space was graded under light microscope according to the model proposed by He et al (Table 1)(6). The grading system was applied by an expert pathologist. In Figure 1, absence of epidural fibrosis is given – grade 0. In Figure 2 and 3 examples of grade I and II are respectively shown. All samples were evaluated en bloc including neighboring tissue but careful attention was shown to visualize the spinal cord, dura mater and epidural space.

**Statistical Analysis**

The data obtained from the study were evaluated using Statistical Package of Social Sciences 15.0 for Windows. Kolmogorov-Smirnov testing was used to test for homogeneity of variables. For descriptive statistics, variables were demonstrated with mean±standard deviation, median (minimum-maximum). Since the results obtained showed homogenous mixture, ANOVA test was used to evaluate to analyze significance in between groups. When analyzing group means Tukey-HSD post hoc analysis was used and p of <0.05 was considered as a significant result.

### Table 1 – Fibrosis Formation Grading System

<table>
<thead>
<tr>
<th>Grade 0</th>
<th>Grade I</th>
<th>Grade II</th>
<th>Grade III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absence of fibrosis over dura mater</td>
<td>Thin fibrous bands between dura and scar tissue</td>
<td>Fibrosis covering less than 2/3 of laminectomy defect</td>
<td>Fibrosis extending to more than 2/3 of laminectomy defect and/or extension to the nerve root</td>
</tr>
</tbody>
</table>

![Hematoxylin-Eosin (HE) stain X 100 sample of absence of fibrosis (Grade 0)](image)
RESULTS

There was no mortality and morbidity related to the procedure, only one of the control group rats had monoplegia lasting for 24 hours. SealFoam® and Surgicel® did not affect the surrounding tissue or wound healing in any of the rats. No complications such as wound infection, hematoma or CSF leakage was observed. All animals were healthy and mobile before euthanasia.

The mean histopathological grade of epidural fibrosis was graded on average 2.12±0.64 in the control group, 0.12±0.34 in the ADCON®-L group, 0.37±0.51 in the SealFoam® group and 0.62±0.51 in the Surgicel® group (Table 2). The control group yielded on average the highest grade while the other groups had less fibrosis.

The difference between the control and ADCON®-L group along with control - SealFoam® and control - Surgicel® was statistically significant (p=0.001). Epidural fibrosis groups 2-3-4 were significantly lower and also the fibrosis grade was significantly lower as well. There was no significant difference of fibrosis in between groups 2-3-4.

<table>
<thead>
<tr>
<th>Specification</th>
<th>mean±SD</th>
<th>median</th>
<th>minimum - maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (laminectomy)</td>
<td>2.12±0.64</td>
<td>2.00</td>
<td>(1.0-3.0)</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laminectomy + ADCON®-L</td>
<td>0.12±0.34</td>
<td>0.00</td>
<td>(0-1.0)</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laminectomy + SealFoam®</td>
<td>0.37±0.51</td>
<td>1.00</td>
<td>(0-1.0)</td>
</tr>
<tr>
<td>Group 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laminectomy + Surgicel®</td>
<td>0.62±0.51</td>
<td>1.00</td>
<td>(0-1.0)</td>
</tr>
</tbody>
</table>

Variable distribution homogenous (p=0.025)

Table 2: The mean histopathological grades of epidural fibrosis amongst groups
Table 3 - Tukey-HSD post hoc Analysis of means in between groups (p=0.001)

<table>
<thead>
<tr>
<th></th>
<th>p 1-2</th>
<th>p 1-3</th>
<th>p 1-4</th>
<th>p 2-3</th>
<th>p 2-4</th>
<th>p 3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.770</td>
<td>0.238</td>
<td>0.770</td>
</tr>
</tbody>
</table>

DISCUSSION

Lower back surgeries are most commonly performed for pain alleviation. Recurrent pain after lower back surgeries is not uncommon and decreases the quality of life for the patient. Although many reasons have been elucidated for failed back surgery syndrome epidural fibrosis is the most common reason\(^5\). The pathophysiology of excessive EF involves mainly the normal healing process – inflammatory cells. Although affected by post operative hemorrhage, infection and surgical technique employed, it is mainly caused by the accumulation of fibroblasts which are transformed to transforming growth factor-1 \(\beta\) (TGF-1\(\beta\)) with the help of proinflammatory cytokines\(^18\).

Intraoperative hemorrhage causes both problems during the operation by blocking the vision of elegant neural tissue and post operatively by causing compression of nerve roots and a prolonged healing process along with excess scarring. Classical methods are employed intraoperatively to achieve hemostasis such as compression, bipolar coagulation and irrigation. Although not novel, polysaccharide hemostatic agents and oxidized regenerated cellulose is also commonly used\(^17\). It is mostly advised to remove these agents once successful hemostasis is achieved. However, most spine surgeons choose not to remove the agents even after hemostasis in the worry of rebleeding in the postoperative period. But no clear side effects are known besides compressing neural tissue when excessive agent is left behind. In recent years, the EF effect of biological agents, autogenous tissue and pharmaceutical agents have been studied.

Local hematoma in the operation field has been proven to increase epidural fibrosis\(^3,15\). Increased platelet aggregation causes platelet-derived growth factor to accumulate causing an increase in myofibroblast migration and proliferation. The mass effect of the hematoma along with increased myofibroblasts hast the potential effect of increasing EF\(^1\). Thus the dilemma of whether to leave behind the artificial hemostatic agent to adequately achieve hemostasis arises.

In this study, post operative epidural fibrosis effect of hemostatic agents SealFoam® and Surgicel® was compared to a control group along with an agent known to decreases fibrosis - ADCON®-L. The control group's EF grade yielded a result of 2.12±0.64 where as ADCON®-L was measured to be 0.12±0.34. The comparison of these two groups was statistically significant (p=0.001) once again proving the effect of ADCON®-L. SealFoam® and Surgicel® had a grade of 0.37±0.51 and 0.62±0.51 respectively. This was significantly lower than the control group and both were statistically significant (p=0.001). Although the fibrosis grades of SealFoam® and Surgicel® groups did not yield statistically significant results when compared with ADCON®-L, the average grade were similar. No statistically significant result was obtained when two hemostatic agents were compared.

Both polysaccharide hemostat and oxidized regenerated cellulose were used to study its effect on epidural fibrosis\(^4,16\). But no research up to date has compared it to an effective agent and used both agents in the same study. This paper yielded similar results to the literature where similar agents have been shown to decrease EF. They did not prove to be
superior to each other but on average had similar results to ADCON®-L.

CONCLUSION

Laminectomy is a widely accepted treatment for lumbar disorders, and epidural fibrosis is a common complication. EF is thought to cause post-operative pain recurrence after laminectomy or discectomy. Post operative hematoma is thought to be the main reason of EF. Whether hemostatic agents cause an increase of fibrosis or prove to beneficial in decreasing EF was the main concept of this research.

This study aimed to study the fibrotic effects of haemostatic agents SealFoam® and Surgicel® compared with ADCON®-L. Although when compared with ADCON®-L these agents did not provide a statistically significant result, when compared with the control group they were both successful in reducing EF. This study indicates that polysaccharide hemostat and oxidized regenerated cellulose do not enhance epidural fibrosis following laminectomy in rats, suggesting that it is not necessary to remove these agents from the surgery field after achieving homeostasis during spinal surgery. More studies are needed to investigate the effects of SealFoam® and Surgicel® on epidural fibrosis.

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