THE EFFECT OF APROTININ ON POSTLAMINECTOMY PERIDURAL FIBROSIS IN THE RABBIT

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The effect of aprotinin on postlaminectomy peridural fibrosis in 20 farm,rabbits was studied in this preliminary study. Laminectomies were performed at two different level (L2 and L4) in 20 rabbits. absorbable sponge soaked with aprotinin or phosphate buffered saline (PBS) was applied in a double-blind fashion to the operative sites in rabbit. Animals were killed after 2 or 4 weeks. The extent of peridural fibrosis was evaluated by gross microdissection and histological analysis. It was shown that aprotinin reduced the peridural fibrosis after laminectomy.

Key Words: *Aprotinin, laminectomy, peridural fibrosis, rabbit.*

INTRODUCTION

The epidural fibrosis at the operative site has been known one of the important cause of failed back syndrome after lumbar laminectomy and discectomy (12). La Rocca and Macnab (5) studied the time course of epidural scar formation histologically and their study indicated fibroblast migration from the adjacent paraspinal muscle. Many synthetic and organic materials have been studied in various animals to investigate for prevention of scar formation. It has been expected that interposing materials would avoid adhesion between dura mater and the scar tissue. Only a few materials have been used in human including gelfoam, methylprednisolone and free fat graft (2, 9).

Aprotinin is well-known agent that lowers the incidence and density of scar formation without any significant side effects(10). aprotinin has been used for prevention of intraperitoneal post-operative adhesions with great success (7, 10). But, there is no study interested in the role of aprotinin to prevent the peridural fibrosis after laminectomy site in the literature.

In this study, the role of aprotinin in the prevention of peridural fibrosis after laminectomy was investigated.

MATERIALS AND METHODS

This experimental study was carried on 20 farm rabbits weighing 2 to 2.5 kg. Ketamine hydrochlorur (Ketalar-Parke-Davis) (35 mg/kg) and xylazine hyrochloride (Rompun-Bayer) (5 mg/kg) were used for sedation. The rabbit was positioned prone on the operating table with the small pad under its abdomen for gaining slight lumbar flexion. The operation performed under open ether anesthesia, following shaving and skin disinfection with povidon-iode. The midline incision was made from L_1 to L_5. The magnification loupes were used during the operation. After, the fascia was incised sharply, the paraspinous muscles were subperiosteally dissected from the spinous process and laminae at two different levels (L_2 and L_4). Total laminectomy with the defect measured about 5x10 mm was performed. Once both laminectomy sites were prepared and excellent hemostasis obtained. The coded samples were placed according to a randomized key. Each coded sample consisted of absorbable gelatin sponge soaked in aprotinin or phosphate-buffered saline (PBS). The fascia overlying the paraspinal muscles closed with 4-0 vicryl sutures in continuous fashion. The subcutaneous tissue and skin were closed anatomically.

Gross evaluation of peridural fibrosis: Animals were killed at the end of 2 (7 rabbits) and 4 (7 rabbits) weeks. The peridural fibrosis at the laminectomy sites were evaluated by microdissection down to the dura mater according to scoring system as shown Table 1.

Histological study: For histological evaluation 6 rabbits were used. The lumbar spines were removed en bloc from L_1 through L_5. The cranial portion was marked with a suture and the specimen was immersed in 10% neutral buffered formalin for approximately 24 hours. Then, the decalcification procedure was done in 5% formic acid for approximately 3 weeks. Two blocks were taken from each laminectomy site to be processed and embedded in parafin. Cross sections 6 μ thick were cut on a cryostat and stained with hematoxylin and eosin. Connective tissue was evaluated with a Masson trichrome stain.

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Table 1: Scoring system for gross evaluation of peridural fibrosis (8).

Score	Description
intermuscular scar	
0	no scar between paraspinal muscles
1	minimal scar tissue
2	moderate scar tissue
3	thick scar tissue
middle scar	
0	no scar present
1	thin layer of scar
2	moderate scar tissue
3	thick scar tissue
deep scar	
0	no scar present
1	thin covering over dura
2	medium covering over dura
3	thick scar tissue
dural adhesions	
0	absent with good anatomical plane
1	moderate adhesion
2	thick and tenacious adhesions
new bone growth	
0	none
1	minimal
2	moderate
3	thick

Figure 1: Control group: Uniformly dense fibrous scarring is evident overlying the laminectomy site. H.E. X 40
f: fibrous tissue, m.s: medulla spinalis.

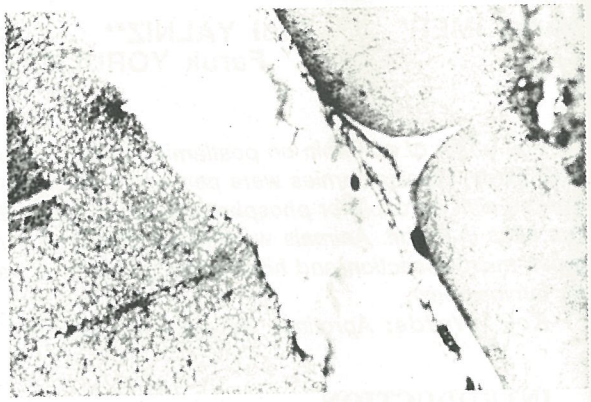
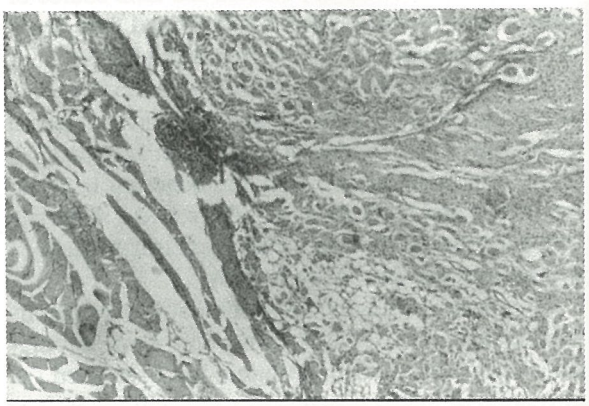


Figure 2: Control group: Dense fibrous scarring is evident in the laminectomy site. Masson's trichrome X 40
f: fibrous tissue, m.s: muscle



RESULTS

Neurologic deficit was not observed in the animals because of the surgical procedure. Four animals died due to anesthetic complications during the operation and they were excluded from the study.

Gross dissection: Fourteen animals were utilized for gross dissection with the aid of magnification loupes. Seven animals were evaluated at the end of 2 weeks and other seven at 4 weeks. The results of dissection demostrated that aprotinin reduced the amount of peridural scar formation following laminectomy at both 2 and 4 weeks using the Wilcoxon nonparametric two-sample rank test (Table 2). The dissection was very easy when aprotinin had been placed at the laminectomy site comparing to

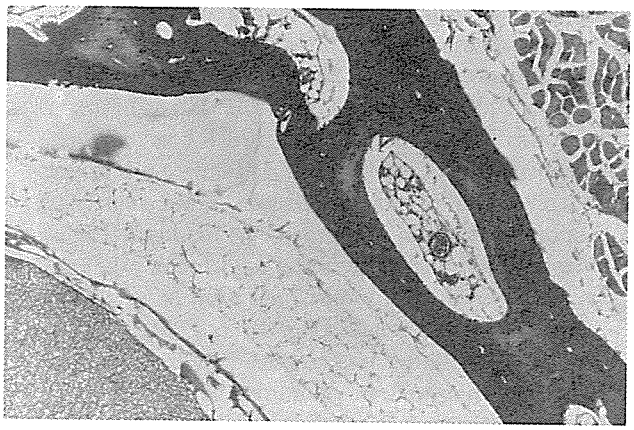
Table 2: Results of gross microdissection in rabbits examined at 2 or 4 weeks.

	2-week group		4-week group	
	Control	Aprotinin	Control	Aprotinin
Intermuscular scar	1.714±0.488	1.429±0.535	2.714±0.488	0.587±0.378
Middle scar	1.714±0.488	1.429±0.535	2.857±0.378	0.714±0.756
Deep scar	2.286±0.756	1.143±0.378	2.714±0.488	1.143±0.378
Dural adhesions	1.429±0.535	0.429±0.535	2	0.571±0.535
New bone growth	2.143±0.69	0.429±0.535	2.429±0.525	1.143±0.378
TOTAL SCORE	13±2.45	6.8±3.56	17.8±2.39	6.2±1.79

the control site. The fibrosis scares in 2 and 4 weeks groups for the aprotinin and PBS-treated groups were compared in Table 2.

Histopathological findings: Six animals were perfused with formalin under anesthesia in order to evaluate the histopathological changes at the laminectomy sites. In this study; light microscopy is used for evaluating the histopathological differences between PBS and aprotinin treated groups. Peridural fibrosis and dural adhesions were very thick at the laminectomy sites in PBS treated control groups (Fig. 1, 2). Also, we noticed dense fibroblastic proliferation at the laminectomy sites in these groups. On the other hand, peridural fibrosis and dural adhesions at the laminectomy sites in aprotinin treated groups were clearly less than the control group (Fig. 3).

Figure 3: Aprotinin treated group: loose areolar connective tissue fills the peridural area. H.E. X 40



DISCUSSION

We have used gross anatomical evaluation and histological analysis to investigate the effect of aprotinin on peridural fibrosis in a rabbit laminectomy model.

The rabbit models of peridural fibrosis have been used extensively (1, 8). The formation of dense fibrosis at the laminectomy site during at least 6 weeks postoperatively in the rabbit is a reliable and qualifies this animal as an appropriate subject for this study (1, 4, 6, 8). Also, the rabbits have low cost and homogeneity among animals of the same size (8).

Boot and Hughes (1) suggested that bone filled the laminectomy defect within 4 weeks in rabbits. In our study; we observed the new bone formation on the laminectomy site, and this was reduced by the aprotinin, especially in the first 2 weeks. However, it is difficult to say that aprotinin reduces the new bone formation.

The fibroblasts may arise from the paraspinal muscles, ligamentum flavum, posterior longitudinal ligament or the annulus fibrosus (4, 5, 6). Therefore, the using materials for study act as a barrier. Robertson et al.(8) has notified that chemoatrectant factors or migration stimulation factors released by the lysis of red blood cells may result in the influx of these scar-forming cells or their precursors. Numerous materials have been placed at the laminectomy site in various animals (1, 2, 4, 5, 8, 12). But, only fre fat graft method has been used extensively in human and still is used by some neurosurgeons (9, 11). The free fat graft is not preventive material for the formation of scar tissue but it plays a role reducing adhesions and provides a good anatomical plane between dura and surrounding tissue (8, 11).

Aprotinin (Trasylol) is a proteinase inhibitor derived from bovine lungs. The mechanism of preventive effect doesn't known exactly. Aprotinin reduces leucocyte infiltration and formation of granulation tissue and hence scar adhesions (10). Aprotinin also has preventive effect on postoperative adhesions in abdominal surgery (3, 7, 10).

Robertson et al. (8) suggested that the rabbit control groups treated with gelfoam plus PBS developed as much peridural fibrosis as did sham sites treated with surgery only (8). We performed the laminectomies at two different level (L_2 and L_4) and absorbable sponge soaked with aprotinin or PBS was applied on laminectomy sites in a double-blind fashion. The results showed that the difference between this two subject was clear.

The gross evaluation of peridural fibrosis revealed that significantly difference between groups (control and aprotinin) occured at 2 weeks after the operation, especially for deep scar and dural adhesions. In the 4-week group; intermuscular scar, middle scar, deep scar, dural adhesions and new bone growth were significantly decreased by aprotinin as shown in Table 2.

Also, the histological finding showed that reduced peridural fibrosis and dural adhesions at the sites treated with aprotinin. As a result, we can say that this effect of aprotinin maintains at least 4 weeks, in this model.

This is the first study with aprotinin in the model of peridural fibrosis, and therefore we couldn't find any other report to compare our results. The preliminary results of this experimental study demonstrated that aprotinin was effective to prevent epidural fibrosis in a rabbit laminectomy model.

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