

Topical Application of Tacrolimus Prevents Epidural Fibrosis in a Rat Postlaminectomy Model: Histopathological and Ultrastructural Analysis

Takrolimusun Sıçan Modelinde Laminektomi Sonrası Oluşan Epidural Fibrosizi Önlemedeki Etkinliği: Histopatolojik ve Ultrastrüktürel Değerlendirme

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ABSTRACT

AIM: Postlaminectomy epidural fibrosis is the formation of scar tissue over the dura mater following posterior spinal surgery. This devastating complication is responsible for the substantial amount of failed back syndromes.

MATERIAL and METHODS: Twenty male Wistar-Albino rats each weighing 350-400 grams were used. Following L3-L5 laminectomy, the rats were randomly divided into 2 groups, with 10 rats in each group. In the control group, only a laminectomy was performed. In the drug group, 5 mg/ml tacrolimus was topically applied with a cotton pad soaked with the drug solution for 5 minutes. The animals were killed on the 30th postoperative day injecting a lethal dose (250 mg/kg) of pentobarbital and the involved dural segments were removed for histopathological and ultrastructural evaluations.

RESULTS: Epidural scar thickness and the density were significantly lower in the animals treated with tacrolimus than those of the control group.

CONCLUSION: Promising evidence regarding the anti-scar potential of tacrolimus merits further research to optimize the dosage and the usage of the drug.

KEYWORDS: Epidural fibrosis, Laminectomy, Tacrolimus, Rat

ÖZ

AMAÇ: Laminektomi sonrası oluşan epidural fibrozis, posterior spinal stabilizasyon cerrahisini takiben dura mater üzerinde yara dokusu oluşumuyla karakterizedir. Bu ciddi komplikasyon, başarısız bel cerrahisi sendromunun da en önemli nedenlerinden biridir.

YÖNTEM ve GEREÇLER: Her biri 350-400 gram ağırlığında yirmi adet erkek Wistar-Albino sıçan kullanılmıştır. L3, L4 ve L5 laminektomiyi takiben sıçanlar sayıları eşit iki gruba rastgele olacak şekilde ayrılmışlardır. Kontrol grubundaki sıçanlara sadece laminektomi uygulanırken, deney grubundaki sıçanlara laminektomi sonrası 5mg/ml takrolimus solüsyonu topikal olarak 1*1 cm'lik pamuk parçalarına emdirilerek 5 dakika boyunca uygulanmıştır. Ratlar operasyon sonrası 30. günde ölümcül doz olan 250mg/kg pentobarbital verilerek öldürülmüş ve ilgili dura segmentleri histopatolojik ve ultrastrüktürel incelemeler için çıkarılmışlardır.

BULGULAR: Epidural yara dokusu kalınlığı ve yoğunluğu takrolimusla muamele edilen sıçanlarda kontrol grubundakilere göre belirgin olarak daha az bulunmuştur.

SONUÇ: Çalışmamızda, saptanan takrolimusun anti-skar etkisi, doz ve kullanım şeklinin belirlenmesi açısından daha ileri çalışmaları gerektirmektedir.

ANAHTAR SÖZCÜKLER: Epidural fibrozis, Laminektomi, Tacrolimus, Sıçan

INTRODUCTION

Postlaminectomy epidural fibrosis is the formation of scar tissue over the dura mater following posterior spinal surgery (3, 9). Additionally, the compression and/or tethering of the nerve roots is a well-known complication of lumbar disc

surgery that may lead to intractable back and leg pain that characterizes the main features of 'failed back syndrome' (1, 3, 9, 10). Minimally invasive surgical techniques are the mostly applied measures to prevent this complication. However, disabling epidural fibrosis may develop in many patients. In

recent years, biological and nonbiological materials have been studied extensively on animals to prevent epidural fibrosis. Some of these materials have been applied in humans, and among these materials, membrane adhesion barrier gels are the most widely applied materials currently (2, 6, 11, 12, 13). However, the significant material cost and the possible need for a repeat surgery seem to be major drawbacks. Tacrolimus (FK-506 or Fujimycin) is a 23-membered macrolactam derivative immunosuppressive drug discovered in 1984 from the fermentation broth of a Japanese soil sample that contained the bacteria *Streptomyces tsukubaensis* (7, 14). It is mainly used for preventing allogeneic organ transplant through reducing the activity of the patient's immune system and so lower the risk of organ rejection. It reduces interleukin-2 (IL-2) production by suppressing T cell function. It is also used in a topical preparation for the treatment of severe atopic dermatitis, severe refractory uveitis after bone marrow transplants, and vitiligo (14, 15).

MATERIAL and METHODS

Animal Population

Twenty male Wistar-Albino rats each weighing 350-400 grams were used. Experiments were conducted in accordance with the National Institutes of Health guidelines and the ethic committee approval was obtained prior to the study.

Surgical Procedure

Anesthesia was achieved by intraperitoneal injection of ketamine (35 mg/kg) and xylazine (10 mg/kg). The lower back of each rat was shaved and the surgical area was sterilized using povidone iodine. A midline skin incision from L-2 to L-5 was performed. The lumbosacral fascia was incised and the paraspinal muscles dissected subperiostally to expose the L3-5 laminae. A total L-3-5 laminectomy was carried out and ligamentum flavum and epidural fat were removed. Hemostasis was managed using gel foam when necessary. The rats were then randomly divided into 2 groups, with 10

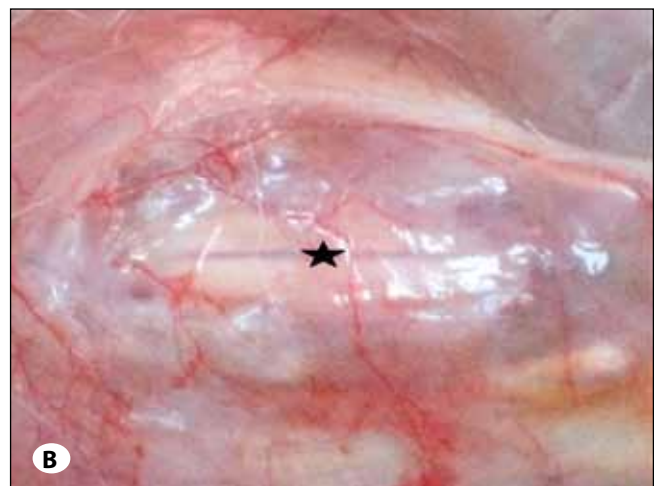
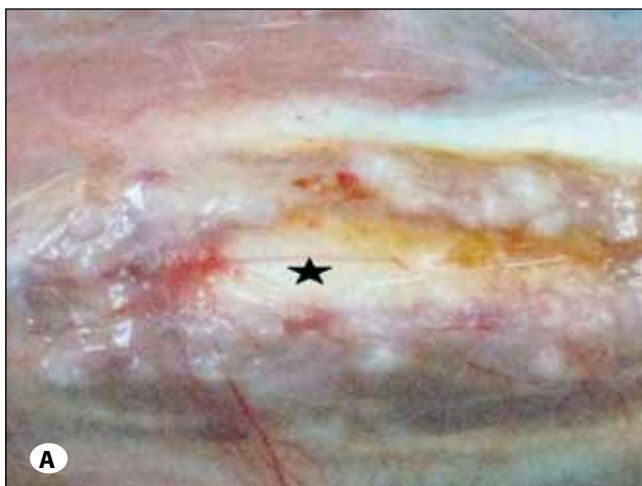


Figure 1A-B: Epidural fibrosis (*) was significantly less in the drug group (A) than that of the control group (B).

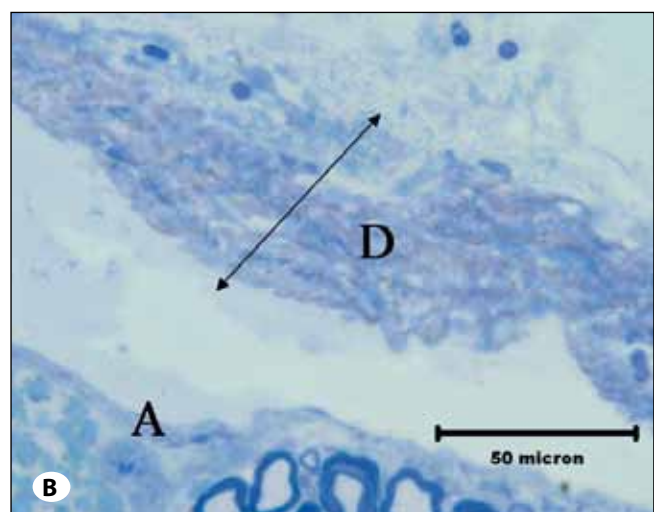
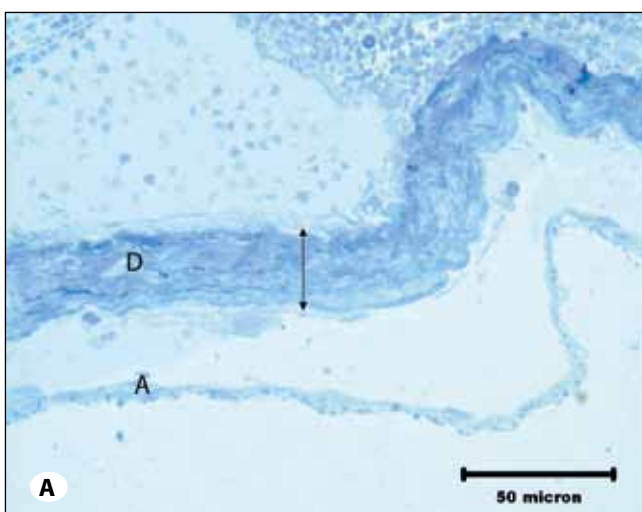


Figure 2A-B: Histopathological examination (Touilidine blue staining) revealed far less epidural scar tissue (Arrow) over the dura (D) in the tacrolimus group (A) as compared to that of control group (B). A: Arachnoid membrane.

rats in each group. In the control group, only a laminectomy was performed. In the drug group, 5 mg/ml tacrolimus was applied with a cotton pad soaked with 0.5 ml of the solution for 5 minutes. The cotton pads were then removed. The wounds were closed in anatomical layers using the same suture material in each animal. There were no complications or adverse effects due to the tacrolimus. The animals were killed on the 30th postoperative day 30 injecting a lethal dose (250 mg/kg) of pentobarbital (IE Ulagay).

Histological and Ultrastructural Examinations

Evaluation of Epidural Fibrosis

The lumbar dura extending between L3 to L5 level were removed en bloc using a circular saw and placed in 10% buffered formalin. After decalcification and dehydration, paraffin blocks were prepared. Axial 8- μ m-thick sections of dura were cut and stained with toluidine blue. Slides were evaluated by the senior pathologist. Specimens were assessed for the density of epidural fibrosis by means of the ratio of epidural fibrosis thickness/epidural fibrosis thickness + dural thickness. For electron microscopic evaluation, the dural tissues segments (10 from the Control Group and 10 from the Tacrolimus Group) were sectioned using an ultramicrotome to a thickness of 30–70 nm. Staining for contrast was performed using uranyl acetate/lead citrate. Stained tissue sections were then examined with a Zeiss electron microscope (Leo 906E).

Statistical Analysis

A paired t-test was used for the determination of statistical significance between the groups for the density of the epidural fibrosis and the p value below 0.05 was set as the significance level.

RESULTS

Gross post surgical observation on the 30th postoperative day revealed that epidural fibrosis was significantly less in the drug group than that of the control group (Figures 1A, 1B respectively). In overall, histopathological and electron microscopic findings revealed that topical application of tacrolimus did effectively reduce the postlaminectomy epidural fibrosis as compared to that of control group ($21 \pm 2.57 \mu\text{m}$ versus $39 \pm 2.17 \mu\text{m}$ respectively). Specifically, Toluidine blue staining revealed far less epidural scar tissue in the tacrolimus group as compared to that of control group ($p < 0.0001$) (Figures 2A,B respectively). Additionally, electron microscopic evaluation of the epidural fibrosis revealed that there are sparsely distributed fibroblasts with large cytoplasm (F), thin fibrocytic processes (*), and collagen fibers (C) in the tacrolimus group (a). On the other hand, there are clusters of fibroblasts (*) due to the increased fibroblast migration and plenty of mitochondria (M) to supply the energy requirement of the activated fibroblasts in the control group (3A,B).

DISCUSSION

Clinically overt epidural fibrosis is a dreadful complication of lumbosacral surgeries. Various biological and synthetic agents for preventing scar formation have been investigated, however, the results were mostly unsatisfactory and clinical applicability of these materials was very limited (2, 6, 10, 11, 12, 13). Currently, the applications of spinal membrane or ADCON gels are the most common measures to prevent postlaminectomy epidural fibrosis (13). These materials are foreign materials and implantation of these implants prolongs operative times. Another drawback of these procedures is that they require second surgery with higher complication

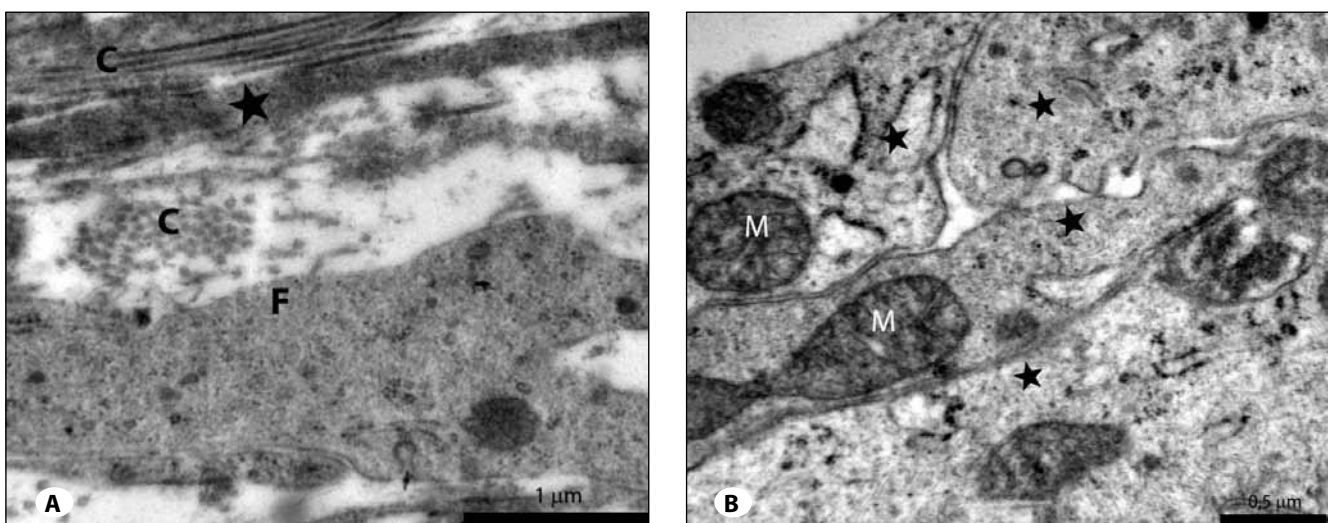


Figure 3A-B: Electron microscopic evaluation of the epidural fibrosis reveals that there are sparsely distributed fibroblasts with large cytoplasm (F), thin fibrocytic processes (*), and collagen fibers (C) in the tacrolimus group (A). On the other hand, there are clusters of fibroblasts (*) due to the increased fibroblast migration and plenty of mitochondria (M) to supply the energy requirement of the activated fibroblasts in the control group (B).

rates than the previous one. There are several agents used for preventing postlaminectomy epidural fibrosis experimentally. Mitomycin C (MMC) is an alkylating antibiotic isolated from *Streptomyces caespitosus* with an anti-fibroblast proliferation (8). It is widely used in ophthalmological surgeries such as trabeculectomy, excision of pterygium, strabismus surgery, and optic nerve decompression. Dogulu et al. reported that topical application of MMC prevented postlaminectomy epidural fibrosis (5). Likewise, Kurt et al. have reported that aprotinin, MMC, and Adcon-L were effective in preventing epidural fibrosis in postlaminectomy rats (13). Collected data in the literature shows that the intraoperative application of MMC is a simple, fast, and inexpensive method in preventing epidural fibrosis (2, 8, 12). However, the effectiveness of the ADCON gels and the other biological membranes and free fat tissue need further research to be determined. Tacrolimus (FK-506 or Fujimycin) is a 23-membered macrolactam derivative immunosuppressive drug discovered in 1984 from the fermentation broth of a Japanese soil sample that contained the bacteria *Streptomyces tsukubaensis*. It is mainly used for preventing allogeneic organ transplant through reducing the activity of the patient's immune system and so lower the risk of organ rejection. It reduces interleukin-2 (IL-2) production by suppressing T cell function (14, 15). In our study, we topically applied tacrolimus solution at the concentration of 5 mg/ml on postlaminectomy dural areas in rats in order to investigate the anti-scar effects of tacrolimus on postlaminectomy rats. The observation period was 30 days. The histopathological and electron microscopic findings revealed that topical application of tacrolimus did effectively reduce the postlaminectomy epidural fibrosis as compared to that of control group ($21 \pm 2.57 \mu\text{m}$ versus $39 \pm 2.17 \mu\text{m}$ respectively). Cemil et al. reported that MMC and pimecrolimus, another compound chemically similar to tacrolimus, prevented the epidural fibrosis in a rat laminectomy model as equally effective (4). As an original contribution, we were able to demonstrate the reduced epidural fibrosis in the tacrolimus group at the ultrastructural level by electron microscopy.

CONCLUSIONS

Our study suggested that tacrolimus is an effective chemical agent in preventing postlaminectomy epidural fibrosis in rats with no clinically overt toxicity. At this point, further research is necessary to enlighten the optimal dosage and the usage of the tacrolimus.

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