

# Comparative Preventive Efficacy of Recombinant Tissue Plasminogen Activator (rt-PA), Urokinase, Hyaluronic acid and Enoxaparin in Post Laminectomy Epidural Fibrosis in Rats

Serdar Kemaloğlu<sup>1</sup>, Ali Gür<sup>2</sup>, Fahri Yılmaz<sup>3</sup>, Hamit Acemoğlu<sup>4</sup>, Ümit Özkan<sup>1</sup>

Dicle University, School of Medicine,  
Departments of Neurosurgery<sup>1</sup>, Physical  
Therapy and Rehabilitation<sup>2</sup>, Pathology<sup>3</sup>,  
and Public Health<sup>4</sup>, Diyarbakır, Turkey

Eur J Gen Med 2011;8(3):170-175

Received: 21.01.2010

Accepted: 04.08.2011

## ABSTRACT

**Aim:** We aimed to determine the effects of rt-PA and enoxaparin on post laminectomy epidural fibrosis and to compare the effects of these two medications with hyaluronic acid and urokinase in an experimental model of spinal epidural fibrosis.

**Method:** Forty Sprague-Dawley rats were anaesthetized by intraperitoneal anesthesia (ketamin 8 mg/100 g) and randomly allocated to five groups. All groups were underwent L1-2 total laminectomies. Topical medications were applied to group 2-5 right after the laminectomy space. Hence, in group 1 (G1=control) only laminectomy was constituted, in group 2 (G2) laminectomy + topical Urokinase, in group 3 (G3) laminectomy + topical rt-PA, in group 4 (G4) laminectomy + topical enoxaparin and in group 5 laminectomy + topical Hyaluronic acid (G5) application were performed. The potentials of local urokinase, rt-PA, hyaluronic acid and enoxaparin in preventing the production of epidural fibrosis were assessed.

**Result:** In inter group comparison, G1-G2, G1-G3, G1-G4, G1-G5 groups differed from each other for total scar score, middle scar score and deep scar score ( $p<0.05$ ). However, G2-G3, G2-G4, G2-G5, G3-G4, G3-G5 and G4-G5 were not different from each other.

**Conclusion:** In rats, the topical thrombolysis with fibrinolytic agents is safe and efficacious in preventing of post laminectomy epidural fibrosis. Thrombolytic therapy with topical hyaluronic acid, rt-PA, urokinase and enoxaparin may come to play an important role in the prevention of post operative spinal epidural fibrosis and arachnoiditis. Based on the results we found that rt-PA and enoxaparin inhibit the formation of epidural scar adhesions and arachnoiditis compared with control group.

**Key words:** Epidural fibrosis, arachnoiditis, rat, urokinase, rt-pa, hyaluronic acid, enoxaparin

**Correspondence:** Serdar Kemaloğlu,  
Faik Ali Sk. Rauf Bey Apt. No:22 Ofis  
21100, Diyarbakır, Turkey  
Tel: 904122288182, Fax: 904122488182  
E-mail: serdarkemaloglu@hotmail.com

### Ratlarda rt-PA, Urokinase, Hyaluronic Acid ve Enoxaparin'in Postlaminektomi Epidural Fibrosisi Önleyici Etkilerinin Karşılaştırmalı İncelenmesi

**Amaç:** Deneysel epidural fibrosis modelinde Rt-pa ve Enoxaparin'in postlaminektomi epidural fibrosis üzerindeki etkinliklerini belirlemeyi ve daha önce bu konuda etkin olduğu yayınlanmış olan hyaluronic asit ve urokinaz'ın etkinlikleri ile karşılaştırmayı amaçladık.

**Metod:** Ketamin ile anestezisi (8mg/100 gr) altına alınan kırk adet Sprague-Dawley cinsi rat randomize 5 gruba ayrıldı. Bütün guruplara L1-2 total laminektomi uygulandı. İlk dört gruba laminektomi alanına ilaçlar topikal olarak uygulandı. Grup 1 de sadece laminektomi, Grup 2 de laminektomi ve topikal Urokinaz, Grup 3'te Laminektomi+Topikal Rt-PA, Grup 4 te laminektomi+topikal Enoxaparin, Grup 5 te laminektomi+topikal hyaluronic asit uygulandı. Topikal uygulanan Hyaluronic asit, Rt-PA, Enoxaparin ve urokinazın epidural fibrosisi önleyici potansiyel etkileri değerlendirildi.

**Bulgular:** Guruplar arası karşılaştırmada G1-G2, G1-G3, G1-G4, G1-G5 total scor, Middle score ve ve Deep Scor bakımından birbirinden farklılık gösterdi(P<0.05). Bununla beraber G2-G3,G2-G4, G2-G5,G3-G4, G3-G5, G4-G5 gruplar arası karşılaştırmada anlamlı bir fark bulunamadı.

**Sonuç:** Fibrinolitik ajanlar ile Topikal trombolitik uygulaması laminektomi sonrası oluşan epidural fibrosisin önlenmesinde güvenilir ve etkindir. Topikal hyaluronic acid, Rt-PA, Urokinaz ve Enoxaparin ile trombolitik tedavi uygulaması ile trombolitik tedavi laminektomi sonrası oluşan epidural fibrosis ve araknoiditisin önlenmesinde önemli bir rol üstlenebilir. Rt-pa ve Enoxaparin'in laminektomi sonrası oluşan epidural skar yapışıklıklarının önlenmesinde kontrol gurubuna oranla etkin olduğunu bulduk.

**Anahtar kelimeler:** Epidural fibrosis, araknoidit, rat, urokinase, Rt-PA, Hyaluronik asit ve enoxoparine

## INTRODUCTION

It is well known that postoperative significant epidural fibrosis and/or adhesive arachnoiditis are one of the most common causes of failed back surgery syndrome after spine surgery however characteristics of patients selected is a very important factor (1-4). Failures of spine surgery characterized by persistent or recurrent pain accounts for up to 40% of all patients after laminectomy and discectomy in long term follow up (1). Adhesive arachnoiditis may occur with any chemical or physical injury to the leptomeninges (1,2,10,16-19). It was also stated that the amount of scar formation after lumbar discectomy related to the clinical outcome, the size of surgical exposure, and some fibrinolytic factors (5). Post operative fibrosis causes nerve root traction that leads to perfusion or conduction disturbances resulting in pain (1).

Up to date there are different studies aiming the prevention of post laminectomy scarring using by different mechanical barriers, chemical regulators of scar formation and prevention of local hematomas (1,3,5,8,10,14-16,18,19,22). Still many substances have been under investigation to remedy the detrimental effects of post laminectomy epidural fibrosis. Hyaluronic acid (1) rt-PA (9) and urokinase (3) have been studied separately and found useful. In the literature we did not find any report investigating the comparative efficiency of antifibrinolytic agents on arachnoiditis and postoperative scar formation. We aimed to evaluate the effects of rt-PA, enoxaparin, hyaluronic acid and urokinase on post laminectomy

epidural fibrosis and arachnoiditis, and to compare their effects in an experimental model of spinal epidural fibrosis and arachnoiditis.

## MATERIALS AND METHODS

Forty Sprague-Dawley rats, aged 6 months, weighing average of 300 g, were utilized in this randomized experimental study. We certify that all applicable institutional and governmental regulations concerning the ethical use of animals were followed during the course of this research. We investigated for prevention of epidural fibrosis on post laminectomy area, and drugs were locally administered in surgical space.

Surgical procedure was performed under intra-peritoneal anesthesia (ketamine hydrochloride 8 mg/100 g). Lower half of the back was sawed and preoperative dose of intramuscularly cefotaxime (100 mg/kg) was given. The rats were positioned prone on the operating table. Above the dorsal area was prepared povidine iodine (Betadine®) soap and solution. The area was draped in an aseptic fashion and a midline incision was made along the spinous process of the lumbar area. The fascia and thoracolumbar muscle were opened bilaterally and total laminectomies of L1-2 were performed. Bleeding was controlled. The dura over the spinal cord was opened and then closed microsurgically. The animals were allocated randomly in the five groups of 8 rats each.

**Table 1.** Scoring system for gross evaluation of epidural fibrosis as total scar score.

Grade 0	When the dura mater was free of the scar tissue.
Grade 1	When only thin fibrous bands between the scar tissue and the dura mater were observed.
Grade 2	When continuous adherence was observed but was less than two-thirds of laminectomy defect.
Grade 3	When scar tissue adherence was large, more than two-thirds of the laminectomy defect, and / or extended to the nerve roots

The first group was the control group in which total laminectomy of L1-2 was achieved and following durotomy dura mater was closed without any medication. In the second group all interventional procedures were done in a similar fashion and Urokinase (25 IU-Laboratories Serono S.A. CH-1170 Aubonne, Switzerland) was inserted beneath and over the dura mater at laminectomy area. Used dose of Urokinase for this study was based on the published knowledge in the literature (3).

**Table 2.** Scoring system for detailed evaluation of epidural fibrosis.

	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 layer of scar
	2	Moderate Scar Tissue
	3	Thick Scar Tissue
Dural Adhesion	0	Absent with good anatomical plane
	1	Moderate adhesion
	2	Thick and tenacious
New Bone Growth	0	None
	1	Minimal
	2	Moderate
	3	Thick

In the third group, after the total laminectomies of L1-2 rt-PA (Actilyse, Boehringer Ingelheim) was inserted beneath and over the dura mater following durotomy in laminectomy area and then dura mater was closed. The rt-PA is supplied as a lyophilized powder that has a concentration of 1 mg rt-PA/ml, pH of 7.3. The dose of rt-PA chosen for this study was 0.25 mg. The chosen dose of rt-PA for this study was based on the published knowledge in the literature (6,7). In the fourth group all interventional procedures were done in a similar fashion and enoxaparin (Clexane® 10 mg/0.1 ml amp, Eczacıbaşı, Istanbul, Turkey; 1.5 mg/kg) was inserted beneath and over the dura mater at laminectomy area. Used dose of enoxaparin for this study was based on the published knowledge in the literature (21). In the fifth group all procedures were done in a similar fashion and Hyaluronic acid (8 mg/kg) (Synvisc 16 mg/2ml, Wyeth, USA) was applied. The chosen dose of Hyaluronic acid for this study was based on the published knowledge in the literature (1). All rats were followed up in a temperature-controlled room for 6 weeks. Neurological function was evaluated weekly. At the end of follow up, the animals were sacrificed by a lethal dose of pentobarbital (60 mg/kg).

Spinal columns were harvested en block immediately after sacrifice and placed in buffered formaldehyde for at least three days. All fixation and dehydration procedure were performed at room temperature. Each specimen was then decalcified with boric acid approximately 3 weeks. Three blocks tissue from each laminectomy site to be processed. Four-micron sections were examined after staining with Haematoxylin and Eosin (H&E). All sections were examined with a light microscope (Olympus BX-50) to measure the tissue response to the two groups. All specimens were evaluated in a blinded manner by two different pathologists. The objective criteria of adhesion were graded according to following classification as Total scar score in Table 1 (22). Additionally we also used scoring system for gross evaluation of peridural fibrosis to investigate the quantity of the peridural fibrosis with another scoring system as stated in previous report in the literature (Table 2) (19).

The histopathological scores for each rat total scar, intermuscular scar, middle scar, deep scar, dural adhesion and new bone growth recorded in the slices of the laminectomy area, and were again retrieved and analyzed. Furthermore, according to the dural adhesion score in the rats; score 0 and 1 were accepted as absence of

**Table 3.** Analysis of the groups with control according to Total Scar Score

Groups	Grading				p
	0	1	2	3	
Urokinase	4/0	3/0	1/7	0/1	0.001
rt-PA	2/0	4/0	1/7	1/1	0.007
Enoxaparin	2/0	4/0	1/7	1/1	0.007
Hyaluronic acid	3/0	2/0	2/7	1/1	0.026

**Table 4.** Analysis of the groups with control according to Intermuscular Scar Score

Groups	Grading				p
	0	1	2	3	
Urokinase	1/0	5/4	2/3	0/1	0.06
rt-PA	1/0	5/4	2/3	0/1	0.06
Enoxaparin	2/0	6/4	0/3	0/1	0.07
Hyaluronic acid	2/0	6/4	0/3	0/1	0.07

arachnoiditis and score 2 were accepted as presence of arachnoiditis. The chi-square test was used to analyze the results of histopathological examination. Significant difference was noted when the probability was less than 0.05.

**RESULTS**

Fourty rats were included in this randomized study program. Rats were divided into five groups to investigate the effects of topical urokinase, rt-PA, enoxaparin and hyaluronic acid in setting which experimental post laminectomy spinal epidural fibrosis. The animals reported in this study were healthy and ambulatory without severe neurological deficit at the time of sacrifice. Three rats died of anesthesia complication in the early post-operative period. Four rats had severe neurological deficit. These rats replaced in the study. Active epidural bleeding was encountered in two rats at the time of surgery and controlled by cotton compression without using bipolar-coagulation. In the control animals, there was severe hemorrhage within surgical area in one rat. One rat had moderate hematoma within surgical area in rt-PA group and two rats had also severe hematoma in urokinase group. These four rats were excluded from the study and four new rats were included instead of them. The incision, subcutaneous tissue, and thoraco-

lumbar fascia of all animals healed without complication, regardless of treatment. Intra-observer reliability were evaluated as for total score 91.7%, intermuscular scar score 81.5%, middle scar score 87.5%, deep scar score 83.3%, dural adhesion 95.8% and new bone growth score 83.3%. Inter-observer reliability of scar scores were 87.5%, 58.3%, 78.2%, 79.2%, 87.5%, 75% respectively.

Comparative results with control and rt-PA, urokinase, hyaluronic acid, enoxaparin for each parameter were showed separately as grade and P values in table 3-8. Inter-groups comparison was statistically performed. In inter group comparison, G1-G2, G1-G3, G1-G4, G1-G5 groups differed from each other for total scar score (p=0.001, p=0.007, p=0.007, p=0.026 respectively). However, comparisons of G2-G3, G2-G4, G2-G5, G3-G4, G3-G5 and G4-G5 for all parameters were made and there were not statistically significant difference from each other. In inter-group comparison, G1-G4 and G1-G5 groups had statistically significant difference for Intermuscular scar score (p=0.07). However, there was not significance between the G1-G2, G1-G3 groups (Table 4). In inter group comparison, G1-G2, G1-G3, G1-G4, G1-G5 groups differed from each other for middle and deep scar (p=0.004 p=0.04, p=0.001, p=0.04 and p=0.001 respectively). And also there were inter group statistical difference between the G1-G2, G1-G3 groups

**Table 5.** Analysis of the groups with control according to Middle Scar Score

Groups	Grading				p
	0	1	2	3	
Urokinase	0/0	6/1	2/7	0/0	0.04
rt-PA	1/0	5/1	2/7	0/0	0.04
Enoxaparin	2/0	6/1	0/7	0/0	0.001
Hyaluronic acid	0/0	6/1	2/7	0/0	0.04

**Table 6.** Analysis of the groups with control according to Deep Scar Score

Groups	Grading				p
	0	1	2	3	
Urokinase	0/0	7/1	1/5	0/2	0.01
rt-PA	0/0	7/1	1/5	0/2	0.01
Enoxaparin	2/0	5/1	1/5	0/2	0.01
Hyaluronic acid	0/0	7/1	1/5	0/2	0.01

**Table 7.** Analysis of the groups with control according to dural adhesion score

Groups	Grading				p
	0	1	2	3	
Urokinase	3/1	4/1	1/4	0/2	0.04
rt-PA	2/1	5/1	1/4	0/2	0.04
Enoxaparin	1/1	5/1	2/4	0/2	0.13
Hyaluronic acid	2/1	4/1	2/4	0/2	0.13

**Table 8.** Analysis of the groups with control according to New Bone Growth Score

Groups	Grading				p
	0	1	2	3	
Urokinase	4/3	1/2	2/2	1/1	1.0
rt-PA	3/3	2/2	2/2	1/1	1.0
Enoxaparin	2/3	4/2	1/2	1/1	1.0
Hyaluronic acid	1/3	3/2	3/2	1/1	1.0

for dural adhesion scar score (p=0.04) (Table 7). But there were any statistical difference between the all groups for new bone formation (Table 8).

The control animals showed heavy scar formation at all laminectomy levels, including thick dural adhesions associated with deep scar (Table 6). The histological observation showed a dens fibrous tissue layer in control defects (Figures 1,2,3). Treatment with all these drugs did not affect the healing of skin subcutaneous tissue or fascia and primarily inhibit peridural scar formation adjacent to the dura and the laminectomy site. We detected lower grade for all evaluated parameters such as middle scar (p=0.04), deep scar (p=0.001) and dural adhesion (p=0.004), total scar (p=0.007) but new bone formation and intermuscular scar were not significantly affected by rt-PA (Table 3-8). (Figures 1,2,3). The presence of arachnoiditis was less in rt-PA and urokinase group (p=0.04).

**DISCUSSION**

Postoperative significant epidural fibrosis and or adhesive arachnoiditis are the one of the most common causes of failed back surgery syndrome after spine surgery (1-4,10,13). Numerous factors are involved in pathogenesis of postoperative epidural adhesions (15). The review of the literature reveals the presence of many hypotheses and treatment attempts regarding epidural fibrosis (3,4,18-20). Epidural fat destruction, local hematoma, migration of fibroblasts from the raw surface of the erector spinae musculature was stated the source of postoperative scar tissue (11-13, 22). Furthermore, foreign body reaction from surgical swab debris, as well as a systemic fibrinolytic defect, has also been reported as possible etiologies of post laminectomy scar formation (4,12). It was also stated that the amount of scar formation after lumbar discectomy

may be related to the size of the surgical exposure, and some fibrinolytic factors (5).

Prevention of post laminectomy scarring has been attempted using different mechanical barriers, chemical regulators of scar formation and prevention of local hematomas (1,3,5,8-10,14,15,17,18,19,22). Still many substances have been under investigation to remedy the detrimental effects of post laminectomy epidural fibrosis. Hyaluronic acid (1), rt-PA (9) and urokinase (3) have been studied separately and found useful on the prevention of post laminectomy epidural fibrosis and arachnoiditis. However, there was no study investigating their comparative responses in post laminectomy epidural fibrosis. Herein we investigated the effects of rt-PA, enoxaparin, hyaluronic acid and urokinase on post laminectomy epidural fibrosis, and compared the effects of these medications. As seen in results section, all medications used in this study were found efficacious on the prevention of post laminectomy epidural fibrosis and arachnoiditis. But we did not find any significant difference among them. For this reason we thought that choosing of preventive medication in spine surgery may be based on the availability and cost of medication.

In conclusion, in rats, the topical thrombolysis with fibrinolytic agents is safe and efficacious in preventing of post laminectomy epidural fibrosis. Thrombolytic therapy with topical hyaluronic acid, rt-PA, urokinase and enoxaparin may come to play an important role in the prevention of post operative spinal epidural fibrosis and arachnoiditis. Based on the results we found that rt-PA and enoxaparin inhibit the formation of epidural scar adhesions and arachnoiditis compared with control group. Any statistical significance between the effectiveness of these medications was found. However, according to the statistical significance level, the efficacies of these medications were ranked as urokinase, rt-PA, enoxaparin and hyaluronic acid respectively.

REFERENCES

1. Abitbol JJ, Lincoln TL, Lind BI, Amiel D, Ing D, Akeson WH, Garfin SR. Preventing post laminectomy adhesion: A new experimental model. *Spine* 1994;19(16):1809-14.
2. Cauchoix J, Ficat C, Girad B. Repeat surgery after disc excision. *Spine* 1978;3:256-9.
3. Ceviz A, Arslan A, Ak HE, İnalöz S. The effect of urokinase in preventing the formation of epidural fibrosis and/or leptomeningeal arachnoiditis. *Surg Neurol* 1997;47(2): 124-7.
4. Dolan RA. Spinal adhesive arachnoiditis. *Surg Neurol* 1993;39:479-84.
5. Dullerud R, Graver V, Haakonsen M, Haaland AK, Loeb M, Magnaes B. Influence of fibrinolytic factors on scar formation after lumbar discectomy. A magnetic Resonance imaging follow-up study with clinical correlation performed 7 years after surgery. *Spine* 1998;23:1464-69.
6. Findlay JM, Weir BKA, Kassel NF, Disney LB, Grace MGA. Intracisternal recombinant tissue plasminogen activator after aneurismal subarachnoid hemorrhage. *J Neurosurg* 1991;75:181-8.
7. Findlay JM, Weir BKA, Steinke D, Tanabe T, Gordon P, Grace MGA. Effect of intratecal thrombolytic therapy on subarachnoid clot and chronic vasospasm in a primate model of SAH. *J Neurosurg* 1988;69:723-35.
8. Jacops RP, McClain O, Neff J. Control of post laminectomy scar formation: an experimental and clinical study. *Spine* 1980;5:223-9.
9. Kemaloglu S, Ozkan U, Yilmaz F, Nas K, Gur A, Acemoglu H, Karasu H, Cakmak E. Prevention of spinal epidural fibrosis by recombinant tissue plasminogen activator in rats. *Spinal Cord* 2003;41(8):427-31.
10. Kuivila TE, Berry JL, Bell GR, Steffee AD. Heparinized materials for control of the formation of the laminectomy membrane in experimental laminectomies in dogs. *Clin Orthop* 1988;236:166-74.
11. Kwan HC, Astrup T. Fibrinolytic activity of reparative connective tissue. *BJ Pathol* 1964;87:409-14.
12. La Rocca H, Macnab J. The laminectomy membrane, studies in its evolution, characteristics, effects and prophylaxis in dogs. *J Bone Joint Surg* 1974;56B:545-50.
13. Lawson KJ, Malucky JL, Berry JL, Steffee AD. Lamina repair and replacement to control laminectomy membrane formation in dogs. *Spine* 1991;16:222-6.
14. Lee JK, Alexander H. Prevention of post laminectomy scar formation. *Spine* 1984;9:305-12.
15. Liu S, Boutrand JP, Bittoun J, Tadie M. A collagen-based sealant to prevent in vivo reformation of epidural scar adhesions in an adult rat laminectomy model. *J Neurosurg (Spine 1)* 2002;97:69-74.
16. Morris GM, Hopewell JW, Morris AD. The influence of methotrexate on radiation-induced damage to different lengths of the rat spinal cord. *Br J Radiol* 1992;65:152-6.
17. Pang D, Scalabassi RJ, Horton JA. Lysis of intraventricular blood clot with urokinase in canine model: part 2, in vivo safety study of intraventricular urokinase. *Neurosurgery* 1986;19:547-52.
18. Quist JJ, Dhert WJA, Meij BP, et al. The prevention of peridural adhesions; a comparative long-term histomorphometric study using a biodegradable barrier and a fat graft. *J Bone Joint Surg [Br]* 1998;3:520-6.
19. Robertson JT, Meric AL, Dohan C, Schweitzer JB, Wujek JR, Ahmad S. The reduction of postlaminectomy peridural fibrosis in rabbits by carbohydrate polymer. *J Neurosurg* 1993;79: 89-95.
20. Tekkok IH, Tekkok S, Ozcan OE, Erben T, Erben A. Preventive effect of intracisternal heparin for proliferative angiopathy after experimental subarachnoid hemorrhage in rats. *Acta Neurochir (Wien)* 1994;127:112-7.
21. Topsakal C, Kilic N, Erol FS, et al. Medroxyprogesterone Acetate, Enoxaparin and Pentoxifylline cause alterations in lipid peroxidation, paraxonase (PON1) activities and homocystein levels in the acute oxidative stress in an experimental model of spinal cord injury. *Acta Neurochirurgica* 2002;144:1021-31.
22. Yucesoy K, Karci A, Kilickap A, Mertol T. The barrier effect of laminae: laminotomy versus laminectomy. *Spinal Cord* 2000;38:442-4.