Original Article

Prevention of spinal epidural fibrosis by recombinant tissue plasminogen activator in rats

S Kemaloğlu^{*1}, U Ozkan¹, F Yılmaz², K Nas³, A Gur³, H Acemoglu⁴, H Karasu¹ and E Cakmak¹

¹Department of Neurosurgery, School of Medicine, Dicle University, Diyarbakir, Turkey; ²Department of Pathology, School of Medicine, Dicle University, Diyarbakir, Turkey; ³Department of Physical Medicine and Rehabilitation, Dicle University, Diyarbakir, Turkey; ⁴Department of Public Health, Dicle University, Diyarbakir, Turkey

Study design: A randomized-controlled experimental study was conducted to investigate the effectiveness of recombinant tissue plasminogen activator (rt-PA) on postlaminectomy epidural fibrosis in rats.

Setting: Diyarbakir, Turkey.

Methods: In a rat model, allowing objective histopathological quantification, the post-operative epidural scar formation was investigated and the potential of local rt-PA in preventing the production of epidural fibrosis was assessed. Rats were randomly allocated to local rt-PA (12 rats) or control (12 rats).

Results: Findings suggest a beneficial effect of rt-PA in decreasing the epidural fibrosis following laminectomy when compared with control groups for all investigated parameters such as intermuscular scar ($P=0.04$), middle scar ($P=0.001$), deep scar ($P=0.001$) and dural adhesion ($P=0.01$) except new bone formation. The presence of arachnoiditis was less in treatment group ($P=0.01$).

Conclusion: In rats, the topical thrombolysis with rt-PA is safe and efficacious in preventing postlaminectomy epidural fibrosis. This Thrombolytic therapy with rt-PA after spine surgery may come to play an important role in the prevention of epidural fibrosis and arachnoiditis.

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Keywords: spinal epidural fibrosis; laminectomy; rt-PA

Introduction

Postoperative significant epidural fibrosis and or adhesive arachnoiditis are the most common causes of failed back surgery syndrome after spine surgery.^{1–4} It is postulated that adhesive arachnoiditis may occur with any chemical or physical injury to the leptomeninges.^{1,2} It is known that local haematoma has the potential to markedly alter the results^{3–6} and increased epidural adhesion may develop because of local haematoma.¹ Platelet-derived growth factor is a mitogene from aggregating platelets responsible for the migration and proliferation of the myofibroblasts. Increased epidural fibrosis by local haematoma may have potential effects by two different mechanisms. Either by a local mass effect of haematoma, or by migration and proliferation of the myofibroblasts generated by platelet-derived growth factor.^{1–3}

Prevention of postlaminectomy scarring has been attempted using different mechanical barriers, chemical

regulation of scar formation and prevention of local haematomas.^{1–4}

This study was designed to assess the effect of topical recombinant tissue plasminogen activator (rt-PA) on epidural scar formation in rats after laminectomy.

Materials and methods

In all, 24 Sprague–Dawley rats, aged 6 months, average weight of 300 g, were utilised in this randomised 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 the postlaminectomy area and rt-PA was locally administered in the surgical area.

The surgical procedure was performed under intraperitoneal anesthesia (Ketalar 8 mg/100 g). A preoperative dose of intramuscularly Cefotaxime 100 mg/kg was given. The rats were positioned prone on the operating table. The dorsal area was prepared

*Correspondence: S Kemaloğlu, Faik Ali Sok. Rauf Bey, Apt. No. 22, Ofis, Diyarbakir 21100, Turkey

using 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 L₂₋₅ were performed. Bleeding was not controlled. The dura over the spinal cord was opened. The animals were allocated randomly in the two groups of 12 rats each.

In the first group, after the total laminectomies of L₂₋₅ rt-PA (Actilyse, Boehringer Ingelheim, Germany) was inserted beneath and over the dura mater following durotomy in the laminectomy area and then the dura mater was closed. The rt-PA is supplied as a lyophilised 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 and was based on the published data in the literature.^{7,8}

In the second group, all procedures were done in a similar fashion but without the insertion of any medication. The 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 killed by a lethal dose of pentobarbital (60 mg/kg).

Spinal columns were harvested *en bloc* immediately after killing and placed in buffered formaldehyde for at least 3 days. All fixation and dehydration procedures were performed at room temperature. Each specimen was then decalcified with boric acid for approximately 3 weeks. Three blocks of tissue from each laminectomy site were processed. Sections 4 µm 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 in the two groups. All specimens were evaluated in a blind manner. The objective criteria of adhesion were graded as in Table 1.⁹ Additionally, we also used a scoring system for gross evaluation of peridural fibrosis to investigate the quantity of the peridural fibrosis with another scoring system as stated in a previous report in the literature⁶ (Table 2).

The histopathological scores for each rat total scar, intermuscular scar, middle scar, deep scar, dural adhesion and new bone growth were recorded in the laminectomy area, and were again retrieved and analysed. Furthermore, according to the dural adhesion score in the rats; scores 0 and 1 were accepted as absence of arachnoiditis and score 2 was accepted as the

presence of arachnoiditis. Interobserver and intraobserver reliabilities were calculated. The χ^2 test was used for statistical analyses. The *P*-values less than 0.05 were assumed statistically significant.

Results

In all, 24 rats were included in this study. Of these, 12 rats were utilised for the study of adhesion formation with rt-PA, the remaining 12 rats were used as a control group. The animals reported in this study were healthy and ambulatory without severe neurological deficit at the time of killing. Only one rat died of anaesthesia complication in the early postoperative period. Active epidural bleeding was encountered in two rats at the time of surgery and controlled by cotton compression without using electrocautery. In the control animals, there was moderate haemorrhage within the surgical area in one rat. In the treatment group, one rat had also moderate haematoma within the surgical area. These two rats were excluded from the study and two new rats were included instead of them. The incision, subcutaneous tissue, and thoracolumbar fascia of all animals healed without complication, regardless of treatment.

Intraobserver reliability was evaluated as for total score 91.7%, intermuscular scar score 81.5%, middle

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

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

scar score 87.5%, deep scar score 83.3%, dural adhesion 95.8% and new bone growth score 83.3%. Interobserver reliability of scar scores were 87.5, 58.3, 78.2, 79.2, 87.5, 75%, respectively. The control animals showed heavy scar formation at all laminectomy levels, including thick dural adhesions associated with deep scar (Table 1). The histological observation showed a dense fibrous tissue layer in control defects (Figure 1). The average adhesion grade in control animals was 2.16 ± 0.38 . Defects treated by rt-PA showed a moderate fibrous tissue layer dorsal to the dura and average adhesion grade was 1.25 ± 1.05 (Figure 2).

Treatment with rt-PA did not affect the healing of skin subcutaneous tissue or fascia and primarily inhibits peridural scar formation adjacent to the dura and the laminectomy site. We detected lower grade for all evaluated parameters such as intermuscular scar ($P=0.04$), middle scar ($P=0.001$), deep scar ($P=0.001$) and dural adhesion ($P=0.001$), total scar ($P=0.001$), but new bone formation was not significantly affected by rt-PA when compared with the control groups (Table 3) (Figure 3). The presence of arachnoiditis was less in the treatment group ($P=0.01$).

Figure 1 Laminectomy site with thick, dense collagenous scar extending into the bone defect (thick arrow) and diffuse attachments to the dura (thin arrow) (H&E $\times 40$)

Discussion

The review of the literature reveals the presence of many hypotheses and treatment attempts regarding epidural fibrosis.^{3,5,6,10} Epidural fibrosis still constitutes an important cause of recurrent symptoms after spinal surgery.^{2-5,10} The Presence of a ruptured disc and operation for its removal are the contributing factors in initiating arachnoiditis. The incidence of arachnoiditis was reported between 10 and 40% after disc surgery.^{3,5} Fibroblasts, originating from the overlying muscles and following extension of postoperative haematoma in to the vertebral canal, cause epidural fibrosis.⁹⁻¹¹ The migration of fibroblasts from the raw surface of the erector spinae musculature was stated as the source of postoperative scar tissue.⁹ Furthermore, foreign body reaction from surgical swab debris, as well as a systemic fibrinolytic defect, has also been reported as possible aetiologies of postlaminectomy scar formation.^{5,12}

Prevention of postlaminectomy scarring has been attempted using different mechanical barriers, chemical regulation of scar formation and prevention of local

Figure 2 Photomicrograph showing section of laminectomy site with moderate fibrous tissue presence in control rat (H&E $\times 40$)

Table 3 Statistical analysis of histopathological examination in rats

Score	<i>rt-PA</i>		<i>Control</i>		<i>Total</i>
	<i>N</i>	%*	<i>N</i>	%*	<i>N</i>
Total scar score					
0	3	100.0	0	0.0	3
1	5	100.0	0	0.0	5
2	2	16.7	10	83.3	12
3	2	50.0	2	50.0	4
			$P=0.001$		
Intermuscular scar score					
0	2	100.0	0	0	2
1	7	63.6	4	36.4	11
2	3	33.3	6	66.7	9
3	0	0	2	100.0	2
			$P^{**}=0.04$		
Middle scar score					
0	1	100.0	0	0	1
1	8	88.9	1	11.1	9
2	3	23.1	10	76.9	13
3	0	0	1	100.0	1
			$P^{**}=0.001$		
Deep scar score					
0	10	83.3	2	16.7	12
1	2	20.0	8	80.0	10
2	0	0	2	100.0	2
			$P^{**}=0.001$		
Dural adhesion score					
0	3	60.0	2	40.0	5
1	7	77.8	2	22.2	9
2	2	20.0	8	80.0	10
			$P^{**}=0.01$		
New bone growth score					
0	5	62.5	3	37.5	8
1	2	33.3	4	66.7	6
2	3	42.9	4	57.1	7
3	2	66.7	1	33.3	3
			$P^{**}=1.0$		
Total	12	50.0	12	50.0	24

*Line percent

**Scores 0 and 1 were united as 0 (absent) and scores 2 with 3 were united as 1 (present) for statistical analyses

haematomas.¹⁻⁴ Inflammation was reported to play a role in the occurrence of scar tissue.¹⁰ Although the occlusion of the laminectomy defect with fat transplants preventing epidural scar formation has been reported, laminectomy defects filled with haematoma resulted in considerable scarring.¹

One of the most important factors protecting from epidural fibrosis is prevention of local haematoma in the operative field. Fibrinolysis in the process of tissue repair has been well described in the literature.¹³ Tissue repair requires the ultimate removal of fibrin accom-

plished either by leucocytic phagocytosis or by fibrinolysis mediated through the local release of plasminogen activator derived from blood vessels.³

Thrombolytic drugs have been widely used for thromboembolic disease and preventing vasospasm caused by subarachnoid haemorrhage.^{7,8,14} It is stated that fibrinolytic treatment in subarachnoid haemorrhage in man appears effective, and may be associated with acceptable risks.^{7,8} Although there was a risk of epidural bleeding with perisurgical epidural use of rt-PA, only one episode of epidural bleeding occurred in rt-PA

Figure 3 Section of a laminectomy site treated with rt-PA showing absence of fibrosis (thick arrow) and dural adhesions (thin arrow) (H&E $\times 40$)

group in this study. This bleeding rate was acceptable for the treatment.

The rats utilised in this study demonstrate reliable formation of postlaminectomy peridural fibrosis at 6 weeks following laminectomy. The application of rt-PA demonstrated a clear reduction in the degree of epidural fibrosis when compared with control group in total score ($P=0.001$), in intermuscular scar score ($P=0.04$), in middle scar score ($P=0.001$), in deep scar score ($P=0.001$), in dural adhesion score ($P=0.01$) and finally in arachnoiditis ($P=0.01$). New bone formation at laminectomy margins was not significantly affected by rt-PA compared with the control groups.

In conclusion, the rt-PA therapy was found statistically significant in experimental postlaminectomy for epidural fibrosis and arachnoiditis in rats. Topical thrombolysis with rt-PA is safe and efficacious in preventing of epidural fibrosis and arachnoiditis. This thrombolytic therapy with rt-PA after spine surgery may come to play an important role in the prevention of epidural fibrosis.

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