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

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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 Spraque-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 fibrinolitic 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
Ratlarda rt-PA, Urokinase, Hyaluronic Acid ve Enoxaparin’in Postlaminektomi Epidural Fibrosisi Önleyici Etkilerinin Karşılaştırmalı İncelenmesi

Amaç: Deneysel epidural fibrosis modellinde rt-PA ve Enoxaparin’in postlaminektomi epidural fibrosis üzerindeki etkileri belirlenmek ve daha önce bu konuda etkin olduğu kaynaklı olan hidrolitik asit ve urokinazın etkilerini ile karşılaştırmak amaçlanmıştır.

Metod: Kafatası ile anestezisi (8mg/kg) altına alınan Kırk adet Spraque-Dawley cinsi rat randomize 5 gruba ayrıldı. Bütün gurularda L1-2 total laminektomi uygulandı. İlk dört gurum subtotal laminektomi altıncı gurunun tam olarak uygulandı. Grup 1 de subtotale laminektomi, Grup 2 de laminektomi ve topikal Urokinaz, Grup 3 te laminektomi-topikal Enoxaparin, Grup 5 te laminektomi-topikal hidrolitik asit uygulandı. Topikal uygulanan hidrolitik asit, rt-PA, Enoxaparin ve urokinazın epidural fibrosis ve araknoiditisin önlenmesinde potansiyel etkileri değerlendirildi.

Bulgular: Guralar arasında karşılaştırıldığında G1-G2, G1-G3, G1-G5 total scar, Middle scar ve Deep Scar bakımından birbirinden farklılık gösterdi (P<0.05). Bununla beraber G2-G3,G2-G4, G2-G5,G3-G4,G3-G5,G4-G5 gruplar arası karşılaştırmada anlamış bir fark bulunulamadı. Sonuç: Fibrolitik ajandalar ile Topikal trombolitik uygulamalar iki cinsiyet epidural fibrosis on klinik veri ve radyoluksal incelemeler ile değerlendirildi. Topikal hidrolitik asit, rt-PA, Urokinaz ve Enoxaparin ile trombolitik tedavi uygulaması ile araknoiditisin önlenmesi konusunda önemli bir rol üstlenebilir. Rekombinant tissue plasminogen activator, urokinase, hyaluronic acid and enoxaparin in post laminectomy epidural fibrosis and arachnoiditis, and to compare their effects with clinical outcome, the size of surgical exposure, and some fibrolytic factors. Post operative fibrosis causes nerve root traction that leads to perfusion or conduction disturbances resulting in pain (1).

INTRODUCTION

It is well known that postoperative significant epidural fibrosis and/or adhesion 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 (1-6). Failures of spine surgery characterized by persistent or recurrent pain accounted 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 fibrolytic 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 anti-fibrolytic agents on arachnoiditis and postoperative scar formation. We aimed to evaluate the effects of rt-PA, enoxaparine, 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

Fourty Spraque-Dawley rats, aged 6 months, weighing average of 300g, 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 located randomly in the five groups of 8 rats each.

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

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No scar between paraspinal muscles</td>
</tr>
<tr>
<td>1</td>
<td>Minimal scar tissue</td>
</tr>
<tr>
<td>2</td>
<td>Moderate scar tissue</td>
</tr>
<tr>
<td>3</td>
<td>Thick scar tissue</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Absent with good anatomical plane</td>
</tr>
<tr>
<td>1</td>
<td>Moderate scar tissue</td>
</tr>
<tr>
<td>2</td>
<td>Thick and tenacious</td>
</tr>
</tbody>
</table>

Table 3. Scoring system for detailed evaluation of epidural fibrosis.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>Minimal</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
</tr>
<tr>
<td>3</td>
<td>Thick</td>
</tr>
</tbody>
</table>

In the third group, after the total laminectomies of L1-2 rt-PA (Actilyse, Boehringer Ingelheim) was injected 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, Eacarecic, Istanbul, Turkey; 1.5 mg/kg) was inserted beneath and over the dura mater at laminectomy area. Used dose of enoxapar ince 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 pentobarbitol (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 dealcified 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-5D) 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 (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

Kemaloglu et al.
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 uroxinase, rt-PA, enoxaparine 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 postoperative 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 uroxinase group. These four rats were excluded from the study and four new rats were included instead of them. The incision, subcutaneous tissue, and thoraco-vertebral fascia of all animals healed without complications, 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, uroxinase, hyaluronic acid, enoxaparine for each parameter were showed separately as grade and P values in table 3-8. Inter-group comparison was statistically perform ed. In inter group comparison, G1-G2, G1-G3, G1-G4, G1-G5 groups differed from each other for total scar score (p=0.001). 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 no 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=0.001, p=0.004, p=0.001 respectively). And also there were inter group statistical difference between the G1-G2, G1-G3 groups for new bone adhesion scar score (p=0.04) (Table 7). But there were any statistical difference between the all groups for new bone formation (Table 8).

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 uroxinase (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 prevention of post laminectomy epidural fibrosis. Herein we investigated the effects of rt-PA, enoxaparine, hyaluronic acid and uroxinase 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 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.
REFERENCES


