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Research Article

Protective Effects of Methotrexate and Tenoxicam in Peridural Fibrosis in Rat Laminectomy Model

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Summary

Objective: Peridural fibrosis after laminectomy is an important surgical problem. Tenoxicam is a nonsteroidal antiinflammatory drug and an oxicam derivative acting by inhibiting the formation of prostaglandin and eicosanoids. Low dose methotrexate which has been used as an antiinflammatory agent rhomatoid arthritis inhibits the synthesis of potentially toxic compounds those accumulate in inflamed tissues. The aim of this study was to evaluate and compare the efficacy of these two independent pharmacological agents in the inhibition of peridural fibrosis.
Methods: L3-4-5 bilateral laminectomy with sparing the dura mater integrity was perfomed to 30 male Wistar albino rats. Except the SHAM group, low dose methotraxate (0.2 mg/kg) or tenoxicam (0.5 mg/kg) was administered on the dura mater before the surgical closure. After 42 days, all rats were euthanized and their spinal columns performed laminectomy were removed for the histopathological evaluation.
Results: The peridural fibrosis grades of the groups administered low dose methotrexate or tenoxicam were lower than SHAM group grades. They have lower fibroblast counts than SHAM group numerically, although there was no statistically significant difference among groups.
Conclusion: Local usage of low dose methotrexate and tenoxicam may be helpful option for prevention of peridural fibrosis after laminectomy procedures in future.

Key words: Peridural fibrosis; laminectomy; methotraxate; tenoxicam

Sıçan Laminektomi Modelinde Metotreksat ve Tenoksikam ile Epidural Fibrozisin Önlenmesi

Özet

Amaç: Laminektomi sonrası epidural fibrozis önemli bir cerrahi problemdir. Tenoksikam prostaglandin ve eikosanoidlerin oluşmasının azaltılmasında rol alan bir oksikam türevi ve steroid olmayan antienflamatuar bir ilaçtır. Düşük doz metotreksat potansiyel toksik bileşiklerin sentezini ve inflamasyon dokusunda bunların birikimini azaltan, yaygın kullanılan bir antimetabolit ajandır. Bu çalışmanın amacı epidural fibrozisin azaltılmasında iki bağımsız farmokolojik ajanın etkinliğinin karşılaştırılması ve değerlendirilmesidir.
Yöntem: 30 erkek Wistar albino rat laminektomi modeli için kullanılmıştır. Dura bütünlüğü korunarak L3-4-5 laminektomi yapılmış, kontrol grubu haricindeki hayvanlara metotreksat (0,2mg/kg) ya da tenoksikam (0,5mg/kg) dura üzerine uygulanmış ve takiben cerrahi yara usulüne uygun şekilde kapatılmıştır. 42 gün sonra tüm hayvanlara ötenazi uygulanmış ve

laminektomi yapılan spinal kolon parçası histopatolojik inceleme için tek parça halinde çıkarılmıştır.
Sonuç: Epidural fibrozis düzeyi düşük doz metotreksat ve tenoksikam uygulanan gruplardaki hayvanlarda kontrol grubundakilerden düşük seviyede tespit edilmiş, ancak fibroblast sayıları bakımından gruplar arasında istatistiksel yönden belirgin fark saptanmamıştır. Yapılan bu çalışma sonunda laminektomi sonrası gelişebilecek epidural fibrozisin önlenmesinde düşük doz metotreksat ve tenoksikamın lokal kullanımının yararlı bir seçenek olabileceği kanısına varılmıştır.

Anahtar Kelimeler: Epidural fibrozis, laminektomi, metotraksad, tenoksikam

INTRODUCTION

Although there are controversial reports, peridural fibrosis after laminectomy procedure is believed to be an important clinical problem because it may lead to dense fibrotic tissue and nerve root adhesions causing chronic low back pain, radicular pain and/ or lower extremity weakness[14]. Adhesion is caused by fibrin accumulation and collagen formation due to inflammatory cell reaction summoning fibroblasts to the tissue[15,18]. Histological studies of experimental adhesion formation have demonstrated the sequence of tissue inflamation, fibrin deposition within an inflammatory exudate, and organization of fibrin with fibroblast invasion and collagen formation, followed by maturation of collagen to produce mature fibrous adhesions[18]. So, this fibroblastic invasion of the peridural area around nerve roots is usually due to a previous laminectomy procedure[5]; and this peridural fibrosis commonly causes persistant postoperative low back pain occasionally accompanied by motor and sensory loss. In case of re-operation, dense scar tissue at the surgical site often renders the dissection of the neural elements from surrounding tissues problematic. Consequently, reccurent surgery is associated with a higher risk of neural injury[1]. Moreover, one of the major factors contributing to the failed back syndrome (incidence rate of 1% to 48%), is postoperative peridural fibrosis[9]. Many animal models were described for the evaluation of various drugs or antiadhesion materials in preventing peridural fibrosis[1,2,5,8,10,13].

Tenoxicam(TNX), an oxicam derivative, is a nonsteroidal anti-inflammatory drug (NSAID) acting by inhibiting the formation of prostaglandin and eicosanoids, which play a major role in the inflammatory response and fibrin production[9]. Previously tenoxicam was administrated systematically in order to decrease peridural fibrosis in rat with unsuccessful results[2]. But, the influence on local delivery of these drugs has not been demonstrated in literature yet.

It has been demonstrated in literature that low dose methotrexate (MTX) has a potent anti-inflammatory activity by inhibiting the synthesis of potentially toxic compounds that accumulate in inflammed tissue[4].

The aim of this study is to investigate the local possible beneficial effects of tenoxicam and low dose methotrexate to reduce or block the peridural fibrosis in a rat laminectomy model.

MATERIAL AND METHODS

Materials

The protocol was approved to be in accordance with the guidelines for the use of laboratory animal subjects in research set by the Ethical Committee of Kırıkkale University, Turkey (Date: Agust 17th, 2010; Number: 10/44).

Thirty male Wistar albino rats with 250-350 gr body weight were randomly assigned into three groups;

- SHAM group (laminectomy was performed but no chemical material was administered; n: 10)

- TNX group (laminectomy was performed and tenoxicam was administered locally; n: 10)

- MTX group (laminectomy was performed and low dose methotrexate was administered locally; n: 10)

Tenoxicam (Tilcotil, Roche, Turkey) was used at dose of 0.5 mg/ kg; and low dose methotrexate (Methotrexat Ebewe; EBEWE Pharma, Austria) was used at dose of 0.2 mg/ kg in this study. Intraperitoneal LD_{50} of the tenoxicam is 401 mg/ kg and the methotrexate is 6 mg/ kg in rats. In clinical experience, low dose methotrexate is administered at dose of 15-17.5 mg weekly to the patients with rheumatoid arthritis[7].

Sedational anaesthesia was performed with intramuscular injection of 40 mg/kg ketamine HCl (Ketalar, Pfizer, Turkey) and 5 mg/kg xylazine HCl (Xylazine 20, Pantex Holland BV, Duizel).

Surgical Technique

After sedational anaesthesia was performed, all animals were immobilized on the operation table in prone position. Following surgical site preparation with povidone iodine application; midline surgical insicion was made from L1 to L5 spinous processes. After detaching the paravertebral muscles from vertebral lamina between L1 and L5 vertebra, laminectomy was perfomed to L3-4-5 segments with care to spare the dura mater integrity (Figure 1)[1,2,5,8,10,17]. In the methotrexate group, 0.2 mg/ kg methotraxate was applied on the dura mater and laminectomy site without any carrier, and the paravertebral muscle and skin layers were closed. The same technique was also applied in the tenoxicam group; and 0.5 mg/ kg tenoxicam was administered directly over the dura mater and laminectomy site without any carrier. In the SHAM group, same surgical steps were performed, but no drug was administered to the laminectomy site. All rats recovered from sedational anaesthesia spontaneously without any problem. Daily observation of the surgical site was performed and uneventful wound healing was noted in all animals for six weeks postoperatively. At 42th day postoperatively, all animals were sacrified via exsanguination by the percutaneous intracardiac route under general anesthesia using ketamine HCl. The whole surgical sites were removed en bloc and fixed in 10% buffered formalin.

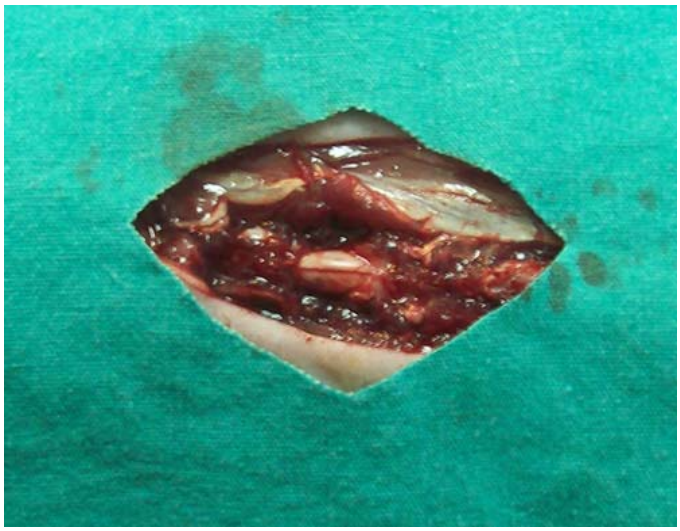

Figure 1: *Figure shows the laminectomy site which was perfomed in L3-4-5 segments with care to spare the dura mater integrity*

Histopathological evaluation

Histopathological evaluation was performed by one observer (S.A.) who was blinded to the treatment arm of the study groups. Prior to the histopathological preperation, after fixation in formaldehyde, the specimens were stored in 10% EDTA solution for 7-10 days in order to obtain adequate decalcification. The tissue blocks contained whole tissue layers including skin, subcutaneous tissue, paravertebral muscles, bone and dura mater-nervous tissue. The specimens were then dehydrated and embedded in paraffin. After preperation of the sections, these sections were stained with Masson-trichrome stain; and hematoxylene and eosin. The stained sections were examined microscopically for evaluation of peridural fibrosis by classifying fibrosis and inflammation in the laminectomy site. Three random areas were assessed in each mm^2 of each section. Peridural fibrosis and adhesions were graded according to the following classification system that was previously described by He et al[10]:

grade 0: the dura mater was free of scar tissue.

grade I: only thin fibrous bands between scar tissue and dura mater were observed.

grade II: continuous adherence was observed but was less than two thirds of the laminectomy defect.

grade III: scar tissue adherence was large, more than two thirds of the laminectomy defect, and/or extended to the nerve roots.

Tissue fibroblast and inflammatory cell counts were classified using the following scale described by Hinton et al[11]:

grade I: the inflammatory cell count is less than 100 in every area on 400x

magnification

grade II: the inflammatory cell count is between 100-150 in every area on 400x

magnification

grade III: the inflammatory cell count is more than 150 in every area on 400x

magnification

Tissue fibroblasts were also counted in three randomly selected areas of the same sections.

Statistical Analysis

All statistical analyses were carried out using SPSS 17.0 statistical software (SPSS Inc., Chicago IL, USA).

The associations between groups were explained as descriptive statistics (n, %) because of inadequate sample size and small frequencies in the cells of cross tables. Variation of fibroblast counts and peridural fibrosis grading scores was not homogenous. These groups were statistically analysed by using the Kruskal-Wallis test. To determine the statistical differences between the groups (post hoc evaluation) the Mann-Whitney-U test was performed to all values.

Inflammatory cell count grades were normally distributed and the variation was homogenous between all groups. Therefore, inflammatory cells count grades were statistically analyzed by the One-Way ANOVA test. Furthermore, to determine the statistical differences between the groups post hoc evaluation (One-Way ANOVA-Tukey Multiple Comparison test) was performed to all values.

p value smaller than 0.05 was considered statistically significant.

RESULTS

Animal Examination

All animals were tolarated the surgical procudures well and they were neurologically intact throughout the study.

Histopathologic analyses results

In the MTX group, four rats had grade I and six rats grade II peridural fibrosis,

while in the TNX group, six rats had grade II and four rats grade III peridural fibrosis. In the SHAM group, all rats had grade III peridural fibrosis (Table 1; Figure 2, and Figure 3). Peridural fibrosis grading scores were statistically significant among the groups ($p<0.001$) (Table 2). The post hoc evaluation results obtained from MannWhitney-U test showed that there were significant differences between SHAM-TNX ($Z=-2.854$; $p=0.023$), SHAM-MTX ($Z=-4.119$; $p<0.001$), and TNX-MTX ($Z=-2.757$; $p=0.015$) groups (Table 3).

Inflammatory cell grading scores

In the MTX group, one rat had grade I and one rat had grade III inflammatory cell density, while remaining rats in the MTX and all rats in TNX and SHAM groups had grade II inflammatory cell density (Table 4, Figure 4). Inflammatory cell grading scores were not statistically different among the groups ($p=1.0$) (Table 5 and Table 6).

Fibroblast cell count results

Although MTX group had lower fibroblast cell counts than other groups numerically, there was no significant difference among groups, statistically ($p=0.146$) (Table 7, Table 8; and Figure 5).

Table 1: Descriptive table of the epidural fibrosis grades (TNX: tenoxicam, MTX: methotrexate)

Groups	n	Grades of Epidural Fibrozis			
		Grade 0(%)	Grade 1(%)	Grade 2 (%)	Grade 3 (%)
TNX	10	-	-	6 (60%)	4 (40%)
MTX	10	-	4 (40%)	6 (60%)	-
SHAM	10	-	-	-	10(100%)

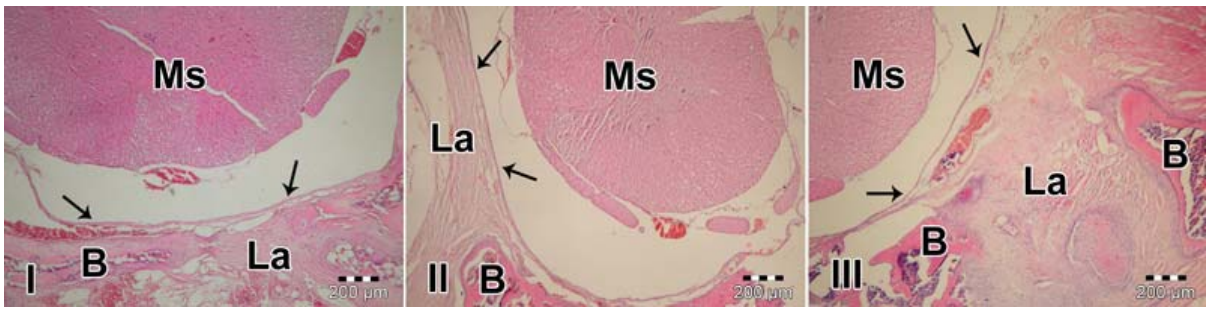

Figure 2: *Histopathological findings of the TNX group (I), the MTX group (II), and SHAM group repectively (III). (H&E; X120). (Arrows shows the dura mater of the rat; B: bone; La: laminectomized area; MS: medulla spinalis)*

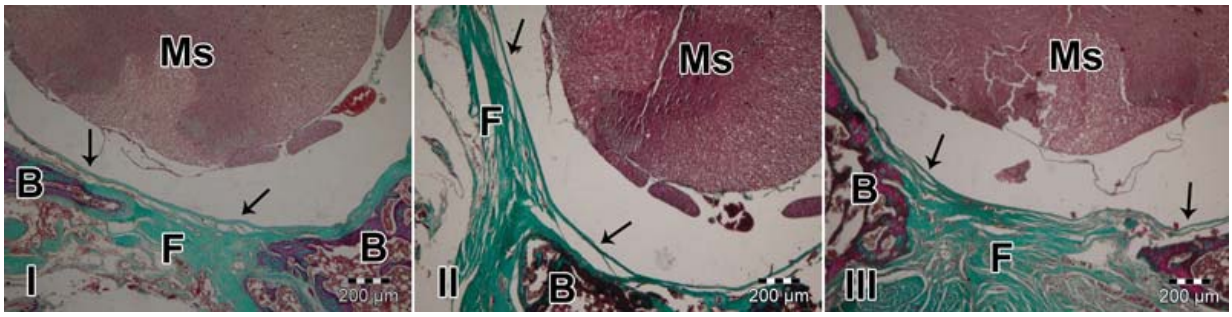

Figure 3: *Histopathological findings of the TNX group (I), the MTX group (II), and SHAM group (III) respectively. (Masson's Trichrome; X120). (Arrows shows the dura mater of the rat; B: bone; F: Fibrous tissue in laminectomized area; MS: medulla spinalis)*

Table 2: Table demonstrates that there was statistically significant difference between all groups described in the text for comparison of their epidural fibrosis grade values. The *Kruskal-Wallis* test, p< 0.05 (MTX: methotrexate, TNX: tenoxicam)

Groups	Mean± Std. Deviation	p*
TNX	2.40±0.52	
MTX	1.60±0.52	<0.001
SHAM	3.00±0.00	

*Kruskal-Wallis test

Table 3: The post hoc evluation results of the epidural fibrosis grading in between groups. The *MannWhitney-U* test, p<0.05 (MTX: methotrexate, TNX: tenoxicam, Z: z score)

Groups	Z	p*
SHAM-TNX	-2.854	0.023
SHAM-MTX	-4.119	<0.001
TNX-MTX	-2.757	0.015

*Mann-Witney U Test

Table 4: Descriptive table of the inflammatory cell grades (MTX: methotrexate, TNX: tenoxicam)

Groups	n	Grades of Inflammatory cells		
		Grade 1	Grade 2	Grade 3
TNX	10	-	10 (100%)	-
MTX	10	1 (10%)	8 (80%)	1 (10%)
SHAM	10	-	10 (100%)	-

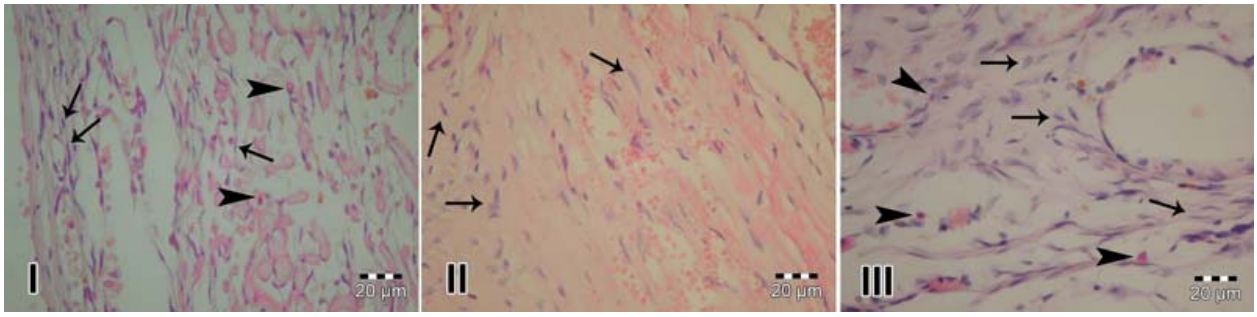

Figure 4: *Fibroblasts (arrows) and inflammatory cells (arrow heads) of the TNX group (I), the MTX group (II), and SHAM group (III). (H&E; X1200).*

Table 5: Table demonstrates that there was no statistically significant difference between all groups described in the text for comparison of their inflammatory cell grade values. The *One-Way ANOVA* test, $p<0.05$ (MTX: methotrexate, TNX: tenoxicam)

Groups	Mean± Standart Deviation	p*
TNX	2±0	
MTX	2±0.471	1.0
SHAM	3±0	

* One-Way ANOVA test

Table 6: This table demonstrates that the variations of the mean values of the inflammatory cell grade values; were not statistically significant between all groups. The *One-Way ANOVA-Tukey Multiple Comparison* test, $p<0.05$ (MTX: methotrexate, TNX: tenoxicam)

Groups	Standart Error	p*
SHAM-TNX	0.122	1.0
SHAM-MTX	0.122	1.0
TNX-MTX	0.122	1.0

* One-Way ANOVA-Tukey Multiple Comparison test

Table 7: Table demonstrates that there was no statistically significant difference between all groups described in the text for comparison of their fibroblast count values. The *Kruskal-Wallis* test, $p< 0.05$ (MTX: methotrexate, TNX: tenoxicam)

Groups	Mean± Standart Deviation	p*
TNX	5947,25±1242,744	
MTX	5026,55±527,126	0.146
SHAM	5920,95±1578,940	

*Kruskal-Wallis test

Table 8: The post hoc evluation results of the fibroblast count results in between groups. The *Mann Whitney-U* test, $p<0.05$ (MTX: methotrexate, TNX: tenoxicam, Z: z score)

Groups	Z	p*
SHAM-TNX	-0.151	0.880
SHAM-MTX	-1.739	0.082
TNX-MTX	-1.626	0.104

*Mann-Witney U Test

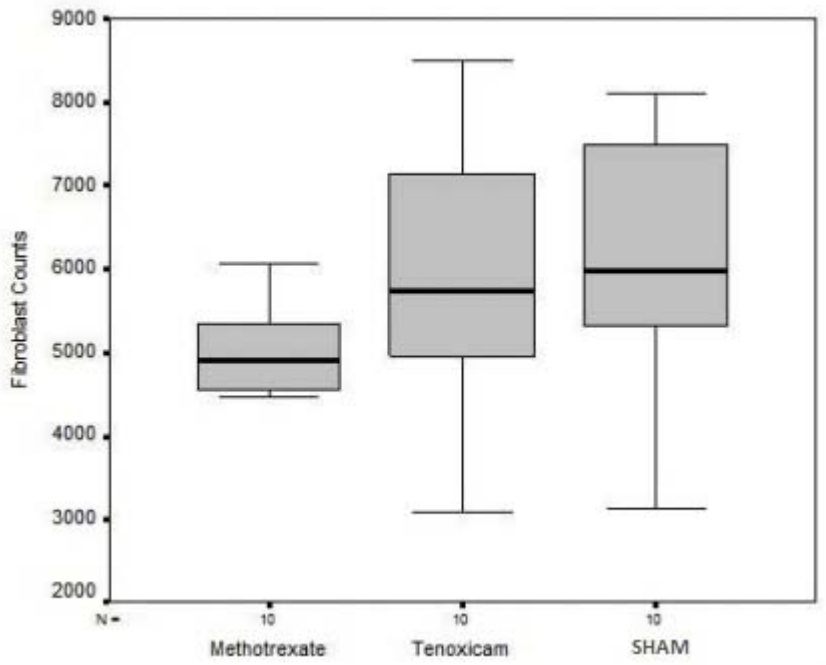

Figure 5: *Although MTX group had lower fibroblast cell counts than other groups numerically, there was no significant difference among groups, statistically.*

DISCUSSION

Peridural fibrosis which may decrease surgical success is a common problem after spinal surgery. The pharmacological basis for adhesion prophylaxis includes agents that decrease the initial inflammatory reaction (such as aprotinin), subsequent exudative release, prevent blood coagulation and fibrin deposition (such as geranylgeranylacetone), and inhibit fibroblastic proliferation (such as mitomycin-C)[7,13,16,19]. In addition, the plasminogen activator inhibitor antagonists may lead to develope an alternative treatment regimen in adhesion prophylaxis, recently[17]. Before it has been documented that tenoxicam which has strong antiinflammatory, antioxidative, analgesic, and antipyretic properties has been succesfully used as a local anti-adhesive drug in abdominal surgery[2,6,20]. Present study demonstrated that tenoxicam administered over the dura mater and its adjacent tissues after performed laminectomy could decrease the peridural fibrosis in a favorable way. In this way, it can be said that its anti-adhesive effect may be originated from its strong antiinflammatory properties, although it could not decrease the fibroblast cell count results effectively. Because this antiinflammatory properties may decrease the back pain related to surgery, it could be recommended to investigate the pain relief effects of the tenoxicam administered into the surgical sites locally in human subjects in future clinical studies, additionally. On the other hand, to reduce the peridural fibrosis grades low dose methotrexate was much more effective than tenoxicam in this model. So, present study revealed that low dose methotrexate also could be acting as an anti-adhesive agent in the rat laminectomy model. Although exact antiinflammatory mechanisms of the methotreaxte have not been identified clearly yet, its anti-adhesive effect may be discussed to be due to a combination of the following mechanisms: 1) Low dose methotrexate leads to a release of high amount of adenosine from the cells which can interact with the A2A receptor on the stimulated inflammatory cells to inhibit cytokine production (e.g. TNF-α) and diminish inflammation[3]. 2) Low dose methotrexate reduces intracellular glutathione concentrations, leading to decreased macrophage functions and accumulation. 3) Low dose methotrexate suppresses T cell activation and adhesion molecule expression[12]. However, our statistical analyses results pointed out that the fibroblast cell count values and inflammatory cell grading values were not different among the groups. Although both experimental durgs could decrease the peridural adhesion efficiently but any of them could not reduce the fibroblast count effectively, we may speculate that methotrexate group had relatively lower fibroblast counts than SHAM and tenoxicam groups, numerically (See Table 7). Thus, further advanced studies are needed to detail these results obviously, and to determine the accurate dose adjusments prior to moving to human studies. But this experimental study has some limitations. First, the experimental drugs could be applied by a carrier (such as an absorbable gelatin sponge) which can offer to be more appropriate as it maintains the drugs and releases them in a slower fashion. But, we thought that a carrier system could possibilty change and confuse our histopathological results, so we did not use these carrier systems. Second, the inflammatory cells located in the adhesive tissue could be distinguished into sub-types (such as neurophils, lymphocytes and monocytes) in this study. But this processes may confuse the study results and their statistical analysis results because of inadequate sample size and small frequencies in the cells of cross tables. In the present study, the evaluation process has been carried out based on the same method which was applied in the

similar studies in literature. Third, because of some financial and technical difficulties of our laboratory facilities, we could not show the far details of the anti-inflammatory mechanisms of these agents (such as not using ultrastructural histopathological analyses, immunohistochemical studies, biochemical analyses, or molecular biological analyses) properly and sufficiently.

CONCLUSION

This experimental study demonstrated that local usage of low dose methotrexate and tenoxicam may be a helpful options for preventing and reducing the peridural fibrosis in laminectomy procedures.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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