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The Effect of Temozolomide on the Prevention of Epidural Fibrosis Developing after Lumbar Laminectomy in Rats

Ratlarda Lomber Laminektomi Sonrası Ortaya Çıkan Epidural Fibrozisin Önlenmesinde Temozolamidin Etkisi

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ABSTRACT

AIM: Failed back surgery syndrome is observed in 15% of patients who have undergone surgery for lumbar disk hernia.Excess epidural fibrosis is the etiology in 24% of FBSS cases. This study was conducted with the belief that the antiproliferative effect of temozolomide can prevent epidural fibrosis.

MATERIAL and METHODS: 8 rats (Group I) underwent laminectomy and were then administered saline while 6 rats (Group II) were administered temozolomide at a dose of 18 mg/kg/day for 5 days after the surgery to make up a total of 14 male Wistar rats used. The pathology preparations of subjects sacrificed at the end of week 6 were histopathologically examined with the Hematoxylin-Eosin stain and Trichrome stain. The pathology preparations were assessed with the analysis parameters and scale generated by He et al. The results were analyzed with the Chi-square test.

RESULTS: No significant difference was found between the two groups in terms of bone and cartilage regeneration, arachnoidal fibrosis, and inflammatory and fibroblast cell densities. Epidural fibrosis formation was significantly less and there was no grade III fibrosis in the Temozolomide group. This was found to be statistically significant (p=0.0302). No side effect of dural or intradural damage was observed.

CONCLUSION: Temozolomide was found to be effective in preventing epidural fibrosis. However, further research is required to determine its effectiveness in local applications and the appropriate dose range.

KEYWORDS: Temozolomide, Epidural fibrosis, Anti-proliferative drug, Laminectomy, Failed back surgery syndrome

ÖZ

AMAÇ: Lomber disk hernisi için cerrahi uygulananların %15'inde başarısız bel cerrahisi sendromu görülmektedir. BBCS etyolojisinin de %24'ünü aşırı epidural fibrozis oluşturmaktadır. Bu çalışma temazolomidin antiproliferatif etkisiyle epidural fibrozisi önleyebileceği düşünülerek yapılmıştır.

YÖNTEM ve GEREÇLER: Laminektomi uygulanıp serum fizyolojik verilen 8 rat (Grup I) ve operasyon sonrası 5 gün boyunca 18mg/kg/gün dozda olacak şekilde temozolomid verilen 6 rat (Grup II) olmak üzere toplam 14 Wistar cinsi erkek rat kullanıldı. 6. hafta sonunda sakrifiye edilen deneklerin patolojik preparatları, Hematoksilen Eozin ve Trikrom boyalarıyla histopatolojik incelemeye alındı. Patolojik preparatlar He ve arkadaşlarının oluşturduğu analiz parametreleri ve skala ile değerlendirildi. Çıkan sonuçlar Ki-kare testiyle değerlendirildi.

BULGULAR: Her iki grup arasında kemik ve kıkırdak rejenerasyonu, araknoidal fibrozis, inflamatuvar ve fibroblast hücre yoğunluğu açısından anlamlı fark saptanmadı. Epidural fibrozis oluşumu ise Temozolomid grubunda belirgin daha azdı ve grade III fibrozise hiç rastlanmadı. Bu istatistiki olarak da anlamlı bulundu (p=0,0302). Yan etki olarak dura ve intradural yapılarda hasarlanma tespit edilmedi.

SONUÇ: Temozolomidin epidural fibrozisi önlemede etkili olduğu saptanmıştır. Ancak özellikle lokal uygulamada etkinliğinin ve uygun doz aralığının saptanması için ileri araştırmalar gerekmektedir.

ANAHTAR SÖZCÜKLER: Temozolomid, Epidural fibrozis, Antiproliferatif ilaç, Laminektomi, Başarısız bel cerrahisi sendromu

INTRODUCTION

Lumbar disk hernia is one of the most common causes of lower back pain and is observed in the general population at a rate of 2-40% with only 15% requiring surgical treatment (11,17,25). Failed back surgery syndrome (FBSS) is observed in 15% of those that undergo surgical treatment (1,20). There are many factors in the etiology of FBSS but approximately 24% of these cases are caused by epidural fibrosis (3). Epidural fibrosis is commonly observed after spinal decompressive surgery but the full clinical picture occurs in only 1-2% of patients (21). The developing fibrosis causes radicular pain by pulling, stretching and generating pressure on the nerve roots. Although there is no problem in the early postoperative period, pain and sensory and motor deficits may appear after 3 to 6 months (2,3,23). The success rate in patients reoperated due to epidural fibrosis ranges between 30-37% and 10-20% of cases have been reported to worsen (4,22). The risk of dural injury and arachnoiditis is also very high in these cases. Epidural fibrosis encountered after lumbar disk hernia or lumbar spinal stenosis surgery constitutes one of the problems that need to be overcome due to its incidence, difficulties in treatment, workforce losses, and the diagnosis and treatment costs.

Many drugs including antineoplastics have been tried to prevent this undesirable condition. Temozolomide is an imidazotetrazinone derivative and it exerts its anti-proliferative effect by stopping the G2/M stage transition in proliferating cells. This study was conducted to evaluate whether the anti-proliferative effect of temozolomide could prevent epidural fibrosis.

MATERIALS and METHODS

This study was carried out at the Gazi University Experimental Animals Research Center after obtaining approval from the medical faculty ethics committee (GUERC). A total of 14 male Wistar rats with weights of 200+/-20 gr were used in this study. The subjects were divided into two groups. Group I (N=8) was the control group that underwent L3 and L4 laminectomy while Group II (N=6) was the group that underwent L3 and L4 laminectomy and then was administered temozolomide at a dose of 18mg/kg/day for 5 days after the surgery (27).

Operating procedure

All surgical interventions were performed under sterile conditions. General anesthesia was achieved with 5 mg/kg xylazine (Rompun, Bayer, Istanbul/Turkey) and 60-100 mg/kg ketamine hydrochloride (Ketalar, Eczacıbası, Istanbul/Turkey). The depth of anesthesia was assessed by giving a painful stimulus from the tail every 15 minutes. Following immobilization of the subjects to the operation table, they were first numbered at the inner surface of their ears. The lumbar region was then shaved. The operation area was sterilized with 10% polyvinylpyrrolidone-iodine mixture. The lumbar fascia was opened after making a midline skin incision approximately 3 cm long over the spinous processes. The paravertebral muscles were subperiosteally dissected from the spinous processes and laminas. The operation site was exposed via small automated retractors. L3 and L4 total laminectomy was performed with a small rongeur and high-speed drill (Aesculap Microtron GD 412, Tuttlingen, Germany) using the surgical microscope (Opmi 99, Carl Zeiss, Germany). The ligamentum flavum and epidural fat tissue were excised. Durameter and nerve roots were exposed (Figure 1). After washing the site with saline, the fascia was sutured with 5/0 vicryl. The skin was sutured with 4/0 silk. The operation site was cleaned again with 10% polyvinylpyyrolidone-iodine mixture. The animals were kept in a room at 28°C for about 30 minutes while they recovered from anesthesia. Loss of strength in the lower extremities was not detected in the postoperative early neurological examinations of the animals. The subjects in Group II underwent the same procedure but were also administered temozolomide at a dose of 18 mg/kg/day for 5 days after the surgery (27).

The rats were sacrificed with the intraperitoneal injection of a lethal dose of sodium pentothal at the end of 6 weeks. The vertebral column was transversely cut approximately 0.5 cm above and below the laminectomy site with a number 20 scalpel while preserving the lumbosacral fascia. The vertebral column was then removed as a block and placed into 10% formalin solution.

Histological study

The lumbar spinal blocks were kept in 10% buffered formalin for 1 week for histopathological analysis. They were then decalcified with 90% formic acid. The vertebral column was macroscopically sectioned at 5mm intervals. Each section was preserved with spinal roots within the dura along the vertebral canal. Sections were paraffin-embedded after tissue processing. 6 µm-thick sections were taken from each slice

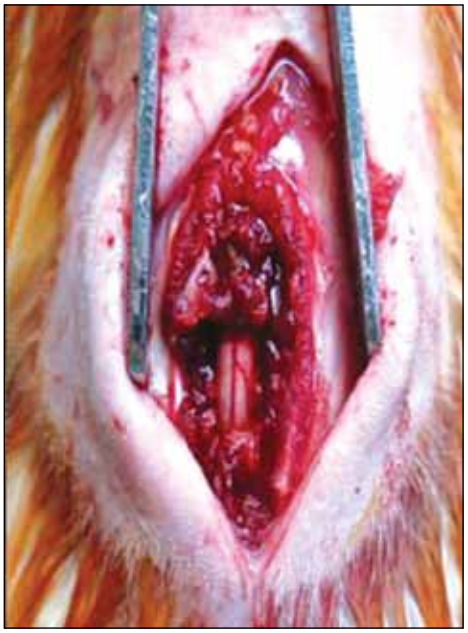

Figure 1: The dura is seen after L3 and L4 total laminectomy, and ligamentum flavum and epidural fat excision.

with the microtome and were subjected to Hematoxylin-Eosin (HE) and Trichrome staining. Epidural fibrosis, fibroblast and inflammatory cell densities, arachnoidal adhesions, bone regeneration, and cartilage regeneration in the area from the lumbosacral fascia to the dura mater and nerve root were evaluated at the Gazi University Faculty of Medicine, Department of Pathology using the analysis parameters and scale generated by He et al. (13).

Statistical methods

The results were compared using the Chi-square test to evaluate whether they were statistically significant.

Result

He and Revel's criteria were used for the assessment conducted together with the Department of Pathology.[13] None of the subjects had a neurological deficit, CSF leakage or infection. No difference was detected for the healing of skin and lumbosacral fascia. No statistically significant difference was found between the groups in terms of bone and cartilage regeneration, arachnoidal fibrosis, and inflammatory and fibroblast cell densities (p=0.94, p=0.59, p=0.78, p=0.797, p=0.485 respectively) (Table I, II, III). In terms of epidural fibrosis, there was a significant difference in the group administered Temozolomide (p=0.0302) where no grade 3 fibrosis not observed. Damage to the dura and intradural structures was not found as a side effect of the medication.

In the control group, grade III fibrosis was found in 4 subjects (50%) and grade II fibrosis also in 4 subjects (50%) (Figure 2). Fibroblast densities were consistent with grade II in 4 subjects (50%) (Figure 3) and with grade I was in the remaining 4 subjects (50%). Five subjects (62.5%) were observed to have significant inflammatory cells (Figure 4) while a level of grade I was observed in 3 subjects (37.5%). Bone regeneration was found in 4 subjects (50%) and cartilage regeneration in 3 subjects (37.5%) Arachnoidal fibrosis was observed in only two subjects (25%) (Table I, II, III).

In the Temozolomide group, grade II fibrosis was determined in 3 subjects (50%) and grade I fibrosis in 3 subjects while

Table I: Histopathological Findings of the Control and Drug Groups. Subjects in Group I are Shown with C and in Subjects in Group II with T

	Fibrosis in Dura mater (Grade)	Fibroblast Density (Grade)	Inflammatory Cell Density (Grade)	Bone Regeneration	Cartilage Regeneration	Arachnoidal Fibrosis	Macroscopic Fibrosis
C1	3	2	1	+	-	+	3
C2	3	2	1	+	+	-	3
C3	2	2	2	-	-	-	2
C4	2	1	2	-	-	-	2
C5	2	1	2	-	-	-	1
C6	3	2	2	+	+	+	3
C7	3	1	1	+	+	-	2
C8	2	1	2	-	-	-	3
T1	1	1	1	-	-	-	1
T2	1	3	1	-	-	-	2
T3	2	2	1	-	-	+	2
T4	1	1	1	-	-	-	1
T5	2	1	2	+	+	-	2
T6	2	2	1	+	+	-	2

Table II: Epidural Fibrosis, Inflammatory Cell and Fibroblast Densities in the Control and Drug Groups

		Grade I	Grade II	Grade III	p
Epidural fibrosis	**Control**	**0.00**	**4 (50%)**	**4 (50%)**	**0.03**
	Temozolomide	**3 (50%)**	**3 (50%)**	**0.00**	
Inflammatory cell density	**Control**	5 (62.5%)	3 (37.5%)	0.00	p= 0.797
	Temozolomide	5 (83.5%)	1 (16.5%)	0.00	
Fibroblast density	**Control**	4 (50%)	4 (50%)	0.00	p=0.485
	Temozolomide	3 (50%)	2 (33.5%)	1 (16.5%)	

Table III: Arachnoidal Fibrosis, Bone and Cartilage Regeneration in the Control and Drug Groups

		(-)	(+)	p
Arachnoidal fibrosis	**Control**	6 (75%)	2 (25%)	0.78
	Temozolomide	5 (83.5%)	1 (16.5%)	
Bone regeneration	**Control**	4 (50%)	4 (50%)	0.94
	Temozolomide	4 (67%)	2 (33%)	
Cartilage regeneration	**Control**	5 (62.5%)	3 (37.5%)	0.59
	Temozolomide	4 (67%)	2 (33%)	

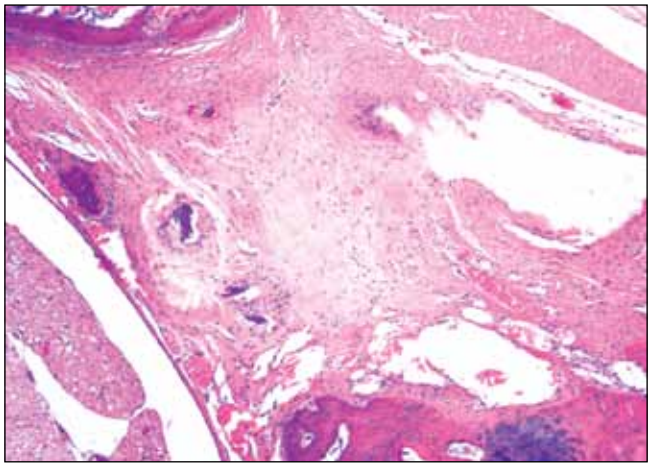

Figure 2: The appearance of grade III fibroblast cell density (Hematoxylin-Eosin stain, 200x).

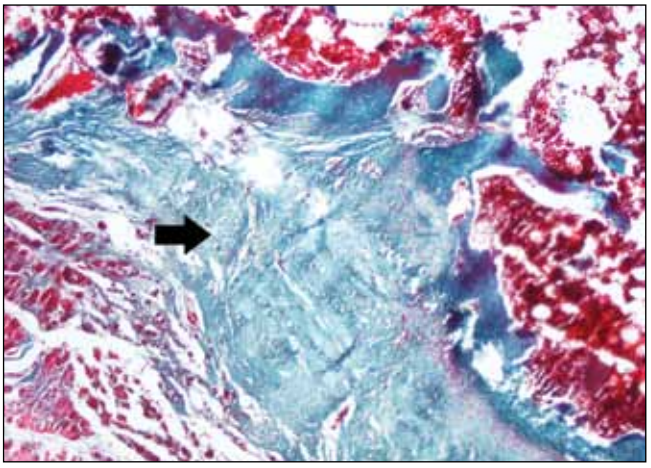

Figure 3: The appearance of grade II microscopic fibrosis (black arrow: epidural fibrosis) (Trichrome stain, 100x).

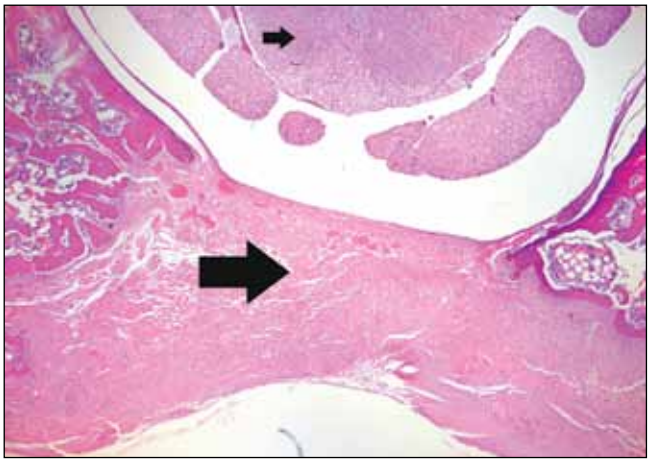

Figure 4: The appearance of grade II inflammatory cell density (Large arrow: area with intense inflammatory cells, Small arrow: medulla spinalis) (Hematoxylin-Eosin stain, 100x).

none of the cases had grade III fibrosis (p=0.0302). Fibroblast density was grade III in 1 subject (16.5%), grade II in 2 subjects (33%) and grade III in 3 subjects (50%). Grade I inflammatory cell density was observed in 5 patients (83%) while grade II was found in only 1 subject (16.5%). Bone regeneration was found in 2 subjects (33%), cartilage regeneration in 2 subjects (33%) and arachnoidal fibrosis in only 1 subject (16.5%).

DISCUSSION

The failed back surgery syndrome is a burden to the economy because of the wide spectrum of treatment methods and the associated large cost. Microsurgery and neuroendoscopic techniques continue to be the most important methods in reducing epidural fibrosis resulting after laminectomy as they cause less tissue damage and reduce the volume of dead-space. Discontented patient profile, treatment difficulties, loss of workforce, and the diagnosis and treatment costs when epidural fibrosis occurs in spinal surgery has led to many studies being conducted on this problem An attempt has been made to decrease fibrosis with existing materials although it has not been possible to completely eliminate it. Successful results have not been achieved at the desired level. Many agents such as nonsteroidal anti-inflammatory drugs (13), gel foam (22), fat grafts, gore-tex (5), carboxymethylcellulose (15), polyactive membrane (6) and ADCON-L (16) have been used in the literature. There are also studies on chemotherapeutic agents that are capable of inhibiting cell division and increasing apoptosis in the treatment of epidural fibrosis. 5-Fluorouracil (24), mitomycin C (8,18,19,26), cyclosporine A (26) are some of the chemotherapeutic agents used in the prevention of epidural fibrosis and reported to have successful results.

Autogenic fat grafts are one of the most commonly used clinical and experimental materials and have been shown

to reduce adhesions by providing a good anatomical plane between the dura mater and surrounding tissue (12). However, it should be noted that 50% of the volume is gradually lost and they may cause symptomatic pressure on nerve roots.

Corticosteroids have been administered topically and systematically by clinicians in order to prevent epidural fibrosis, to reduce the inflammatory cell response and also to increase the activity of collagenases (10). However, there is insufficient evidence in the literature about steroids regarding prevention of epidural fibrosis as far as we know. He and Revel reported 40% less epidural fibrosis in the treatment group with ketoprofen, a non-steroidal anti-inflammatory drug (13).

Low-dose radiation therapy has been reported to reduce the extent, density and adhesiveness of post-operative scar tissue (3).

Temozolomide was synthesized within a series of modified imidazotetrazines by Stevens et al. in 1987 (7). It establishes its cytotoxic effect especially by the transformation of guanine at the O6 position by methylation to O6-methylguanine. The cell stops at the G2/M phase transition and undergoes apoptosis as a result (7,14). Inflammation and regeneration are interconnected and we therefore investigated the effectiveness of temozolomide, postulating that connective tissue formation can be limited by blocking cell proliferation.

Histological criteria including epidural fibrosis, fibroblast and inflammatory cell densities, arachnoidal adhesion, bone regeneration and cartilage regeneration as proposed by He et al. were used in our study to objectively evaluate epidural fibrosis in accordance with the literature (13). A statistically significant difference was found between the two groups when the extent of fibrosis in the dura mater was assessed ($p=0.0302$). The formation of epidural fibrosis was significantly less in the Temozolomide group and no grade III fibrosis was found as shown in Table 2. No statistically significant difference was detected with the control group for arachnoidal fibrosis, inflammatory cell density and fibroblast density. We believe that it is not possible to completely neutralize regeneration as it is the most important defense mechanism of all organisms against internal and external factors. However, it is possible to reduce dysfunctional scar formation. Similar results were obtained in the literature with a study using mitomycin-C which is an anti-chemotherapeutic similar to temozolomide in terms of the mechanism of action (8).

Bone regeneration has been shown in the laminectomy defect, just like peridural fibrosis, in the majority of experimental studies. He and al. have reported that formation of new bone starts from the laminectomy site and osteoblasts are activated (13). Cook et al. have reported that bone regeneration takes place at a rate of 50% in 8 weeks and 75% in 12 weeks (6). Einhaus et al. have found a 5-10% reduction in the laminectomy defect at the end of 4 weeks in their study (9). No significant differences were found with the control group when examining bone and cartilage regeneration in our study. Another important point is that dura mater and intradural tissues were not damaged as a side effect of temozolomide.

The effectiveness of temozolomide, which has recently become popular in the treatment of high-grade glial tumors, has been shown in experimental epidural fibrosis with the data we obtained. The safe and effective dose range of temozolomide that we administered orally to our subjects who had undergone laminectomy in our study should be determined. This agent has superior properties in the fight against fibrosis but it can block mitosis in surrounding normal tissue cells. It may also lead to necrosis and endothelial injury. In light of these factors, more common use in experimental applications and an increase in the number of test subjects are inevitable. Another negative factor is the high cost of the material at the moment.

Completely preventing fibrosis carries a risk in terms of the problems it may cause as it is necessary for tissue repair and does not seem possible. However, microsurgical intervention to reduce excessive scar formation, good hemostasis and early mobilization are considered the most effective methods in preventing epidural fibrosis. Microsurgical intervention limits the inflammatory response by causing minimum damage in the paravertebral muscles, ligamentum flavum and epidural fat tissue. Furthermore, microsurgical intervention should also be preferred as it causes less tissue loss, considering that the more the tissue loss the more the fibrosis that develops. The surgical site must be closed after good hemostasis as the inflammatory response of blood products increases fibrosis. Mobilizing patients in the early period may also be effective in reducing the severity of fibrosis due to its positive impact in terms of the quantitative and qualitative properties of wound healing.

CONCLUSION

We believe that temozolomide, an antiproliferative and antineoplastic agent used in the chemotherapeutic treatment of high-grade glial tumors, will be effective in the treatment of epidural fibrosis. The safe and effective dose range of temozolomide that we administered orally to our subjects for patients who had underwent laminectomy should be determined. This agent has superior properties in the fight against fibrosis but it can block mitosis in surrounding normal tissue cells. It is therefore necessary to conduct more experimental studies and determine its effective dose, the best administration route and clinical significance, if any.

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