Received: 13.02.2012 / **Accepted:** 06.03.2012
DOI: 10.5137/1019-5149.JTN.6008-12.0

Prevention of Epidural Fibrosis in Rats by Local or Systemic Administration of Citicoline

Sıçanlarda Lokal ve Sistemik Sitikolin Uygulanması ile Epidural Fibrozisin Önlenmesi

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ABSTRACT

AIM: The objective of this study was to investigate the effect of citicoline administration on epidural fibrosis which is a frequent complication of lumbar disc surgery with no effective treatment or preventive surgical technique.

MATERIAL and METHODS: Sixty Sprague-Dawley female rats undergoing L4-5 right hemilaminotomy and annular fenestration were arranged in three groups: rats in Group 1 (control group) and Group 2 (topical citicoline group) were applied 0,9% saline and 100 µM citicoline on surgical area, respectively, while rats in Group 3 (systemic citicoline group) received 600 µmol/kg citicoline intraperitoneally. Rats were sacrificed four weeks later and their vertebral colons were removed en bloc. Groups were evaluated according to histological criteria and results were compared using statistical tools.

RESULTS: Compared with control group, significantly less epidural fibrosis, dural adhesion, fibroblast cell density, foreign body reaction, and medulla spinalis retraction were observed in groups treated with topical and systemic citicoline (groups 2 and 3) ($p<0,001$). No significant difference was found with regard to measured parameters between two treatment groups ($p>0,05$).

CONCLUSION: Our study demonstrates for the first time in the literature that citicoline may be effective for preventing postoperative epidural fibrosis. However, its mechanism of action and clinical effectiveness must be further investigated.

KEYWORDS: Epidural fibrosis, Annular fenestration, Laminatomy, Citicoline, Rat

ÖZ

AMAÇ: Çalışmamızın amacı, sitikolin uygulamasının henüz efektif bir tedavisi veya koruyucu bir cerrahi tekniği bulunmayan ve lomber disk cerrahisinin sık bir komplikasyonu olan epidural fibrozis üzerine etkisini incelemektir.

YÖNTEM ve GEREÇLER: Sağ L4-5 hemilaminatomive anular fenestrasyonu yapılmış 60 Spraque Dawley dişi sıçan 3 gruba ayrıldı. Grup 1 (kontrol grubu) ve Grup 2 (topikal sitikolin grubu)'deki sıçanlara cerrahi alan üzerine sırasıyla 0,9% serum fizyolojik ve 100 µM sitikolin uygulanırken, Grup 3 (sistemik sitikolin grubu)'deki sıçanlara intraperitoneal yolla 600 µmol/kg sitikolin verildi. Sıçanlar 4 hafta sonra öldürüldü ve vertebral kolonları blok olarak çıkarıldı. Gruplar histolojik kriterlere göre değerlendirildi ve sonuçlar istatistiksel yöntemler kullanılarak karşılaştırıldı.

BULGULAR: Topikal ve sistemik sitikolin ile tedavi edilen (Grup 2 ve 3) gruplarda kontrol grubuna kıyasla istatistiksel olarak daha az epidural fibrozis, dural yapışıklık, fibroblast hücre yoğunluğu, yabancı cisim reaksiyonu ve medulla spinalis retraksiyonu gözlendi ($p<0,001$). İncelenen parametreler bakımından iki tedavi grubu arasında anlamlı bir fark bulunmadı ($p>0,05$).

SONUÇ: Çalışmamız literatürde ilk kez sitikolin'in postoperatif epidural fibrozisi önlemede efektif olabileceğini göstermiştir. Ancak sitikolinin etki mekanizmaları ve klinik etkinliği ilerideki çalışmalarla araştırılmalıdır.

ANAHTAR SÖZCÜKLER: Epidural fibrozis, Annular fenestrasyon, Laminatomi, Sitikolin, Sıçan

INTRODUCTION

Surgical dissection into the spinal canal is challenging during lumbar revision surgery in cases with epidural fibrosis since it increases such intraoperative complications as occurrence of dural tears, spinal nerve lesions, and local bleeding (3, 34, 35, 39, 46). Dense scar tissue increases the morbidity of subsequent operations to the same region (11, 26, 43, 45). Braverman et al., reported that repeated surgery for fibrosis had only a 30% to 35% success rate (9), whereas 15% to 20% of patients reported worsening of their symptoms (33).

Various surgical procedures including minimally invasive surgery with small incision, limited manipulations during surgery, proper hemostasis and removing foreign materials from epidural region, placing mechanical barriers within dura

and adjacent tissue have been proposed to reduce epidural fibrosis (3, 20, 37). In addition, numerous materials and drugs have been used experimentally and clinically to prevent epidural fibrosis (8, 10, 16, 18, 20, 22, 31, 32, 46, 56). However, epidural fibrosis still remains to be a major clinical problem in patients undergoing surgery for disc herniation.

Cytidine 5'-diphosphate choline (CDP-choline) is an endogenous intermediate in the biosynthesis of cell membrane phospholipids and exogenously-administered CDP-choline is referred to as citicoline (53). Experimental and clinical studies reported beneficial use of citicoline in neurodegenerative diseases such as Alzheimer's and Parkinson's disease, in improving learning and memory, acute and chronic cerebrovascular diseases, traumatic brain injury and glaucoma (30, 47, 48).

Data obtained in our previous studies on citicoline's effects on promoting regeneration of peripheral nerves subjected to immediate section suturing type surgery and reducing postoperative scarring, and recent similar observations encouraged us to test citicoline in prevention of epidural fibrosis after lumbar disc surgery (6, 12, 36). Hence, the purpose of our study was to investigate local and systemic effectiveness of citicoline on epidural fibrosis in a rat model of laminotomy and discectomy.

MATERIAL and METHODS

Rat population and Surgical Technique:

Our study was approved by the Animal Research Ethical Committee of Uludag University. We used 60 female Sprague-Dawley rats weighing 200-250 grams. Each animal received one dose intramuscular (i.m.) injection of cephazolin sodium (20 mg/kg) for prophylaxis. Rats were anesthetized with ketamine hydrochloride (70 mg/kg; i.m.) (Ketalar, Pfizer-Turkey, 2007) plus Xylazine hydrochloride (10 mg/kg; i.m.) (Rompun, Bayer, Turkey). After stabilizing rats on the operation table in prone position, the surgical site was brushed with ChlorHex-OL and sterilized with povidone-iodine solution. Bilateral paraspinal muscles were subperiosteally dissected following a lumbar midline incision. Under operation microscope (Opmi, CARL ZEISS, Germany) the L3–5 laminae were exposed and a right hemilaminectomy were performed at L-4 level using high-speed drill. Dura mater was exposed by removing epidural fat tissue. After finding disc space annular fenestration were performed by using 22G catheter. Hemostasis was maintained by irrigation with saline and surgical gauze throughout the surgical procedure. The hemilaminectomy site was marked with 4-0 vicryl in all rats and the layers were closed. Rats were divided into three groups. Group 1 (control group, n=20) underwent L4-5 right hemilaminotomy and annular fenestration, and spongostan soaked in 0,9% saline was applied to surgical area. In Group 2 (topical citicoline group, n=20) spongostan soaked in citicoline (100 µM) was applied to surgical area locally after identical surgical procedure. In Group 3 (systemic citicoline group, n=20) citicoline was applied intraperitoneally at a dose of 600 µmol/kg after identical surgical procedure.

Preparation of Specimens:

Four weeks after the surgery rats were killed by ether inhalation. For histopathologic studies, the lumbar spine including the surgical site was removed en bloc. The specimen was immersed into 10% buffered formalin solution. Each specimen was then decalcified in equal quantities of 10% formic acid and 8% hydrochloric acid solution for 3 days. All specimens were then washed with tap water for 12 hours. Five ±m thick serial sections were cut from formalin-fixed paraffin-embedded tissues and stained with hematoxylin and eosin (HE) and Masson trichrome (MT).

Histopathological Evaluation:

All specimens were examined microscopically by the same investigator who was blinded to the study. The density of epidural fibrosis, dural adhesion, foreign body reaction, fibroblast cell density and medulla spinalis retraction were investigated. The density of epidural fibrosis, foreign body reaction, and medulla spinalis retraction were scored as absent (0), mild (1), moderate (2), or severe (3) as described previously (49) (Table I).

Dural adhesions were scored as follows: grade 0, the dura is free of scar tissue; grade 1, thin fibrous bands are observed between the dura and scar tissue; grade 2, continuous adherence is observed in less than two-thirds of the laminectomy defect; grade 3, adherence is observed in more than two-thirds of the laminectomy defect, or extends to the nerve roots as described previously (23) (Table II).

Grading of the fibroblast cell density was made as follows: grade 1, less than 100 fibroblast cells per 400x field; grade 2, 100-150 fibroblast cells; grade 3, more than 150 fibroblast cells as described previously (24) (Table III).

Statistical analysis:

Statistical analyses were performed using SPSS 13.0 (Chicago, IL.) software. Median minimum and maximum values were granted for the density of epidural fibrosis, dural adhesion, foreign body reaction, fibroblast cell density and medulla spinalis retraction. Comparisons between groups were made using Kruskal Wallis test. In subgroup analysis, groups were compared in doubles using Mann- Whitney U test and p value <0.05 was considered statistically significant.

RESULTS

Four rats died during the study: two of them due to anesthetic complications and the other two due to dural injury. These 4 animals were replaced in order to achieve a constant number of rats in all three groups.

All rats recovered without wound infection. Similarly, no signs of infection were noted in subcutaneous tissue, fascia, and paravertebral muscles.

Group 1 (control group) showed dense epidural scar formation at laminectomy defects (Figure 1, 2A-C). Compared with group 1, significantly lower values were obtained with

Table I: Scoring of the Density of Epidural Fibrosis, Foreign Body Reaction, and Medulla Spinalis Retraction

Grade 0	Absent
Grade 1	Mild
Grade 2	Moderate
Grade 3	Severe

Table II: Grading of the Extent of Dural Adhesions

Grade 0	The dura is free of scar tissue
Grade 1	Thin fibrous bands are observed between the dura and scar tissue
Grade 2	Continuous adherence is observed in less than two-thirds of the laminectomy defect
Grade 3	The adherence is observed in more than two-thirds of the laminectomy defect, or extends to the nerve roots

Table III: Grading of the Fibroblast Cell Density

Grade 1	Less than 100 (400x)
Grade 2	100 – 150 (400x)
Grade 3	More than 150 (400x)

regard to epidural scar formation (p<0,001 and p<0,001), dural adhesion (p<0,001 and p<0,001), fibroblast cell density (p<0,001 and p<0,001), foreign body reaction (p=0,021 and p=0.013) and medulla spinalis retraction (p=0,043 and p=0.033) in group 2 (topical citicoline group) and group 3 (systemic citicoline group), respectively. On the other hand, considering all parameters measured, no significant differences were found between topical citicoline (group 2) and systemic citicoline (group 3) groups (p>0,05). Grading scores of all parameters in three groups and comparative graphics of mean values are shown in Table IV and Figure 1, respectively.

DISCUSSION

These data show that citicoline treatment significantly reduces epidural scar formation, dural adhesion, fibroblast cell density, foreign body reaction and medulla spinalis retraction in a rat model of laminotomy and discectomy. Similar beneficial effects are observed by either local or systemic administration.

Epidural fibrosis is usually observed post-spinal surgery as nonphysiological scar formation around the origin of the radicular sheath. It behaves as a reparative inflammation causing clinical problems of characteristic nature (5, 32, 51, 52). Epidural fibrosis affects the normal neural dynamics of nerve root by producing adhesions tethering the nerve root to adjacent tissues. Root ischemia caused by tethering may be an important contributor to the generation of radicular pain (44).

Discectomy (annular fenestration) was not performed in most experimental studies that evaluated postoperative scar formation, because of the belief that epidural fibrosis derived from posterior spinal muscles. However, it was reported that nucleus pulposus, which appears in peridural region due to annular fenestration, could take part in the formation of peridural fibrosis and in the etiology of pain by inflammatory immunological effects and by causing microvascular changes (43). Therefore, we used laminotomy and discectomy model in our study to investigate the effect of citicoline on postoperative scarring.

Attempts have been made for prevention of epidural fibrosis after lumbar disc surgery, and, to date, many materials have been used in this regard (3, 8, 22, 29). Despite encouraging results obtained in experimental studies, no mechanical or chemical intervention has proved to be beneficial in the clinical setting so far. Therefore, preventing post operative epidural fibrosis continues to be a clinical problem.

Fat grafts were the first to be tested, and currently they are the most commonly used materials. Unfortunately, these grafts showed little benefit; they reduce tethering rather than preventing epidural fibrosis (22, 40). Numerous materials and strategies including low dose external beam radiation, a carbohydrate polymer, ADCON-L, non-steroidal anti inflammatory drugs (NSAID), anticancer agents such as mitomycin C, cyclosporin A, and 5-fluorouracil, topical trombolysis with recombinant tissue plasminogen activator (r-tPA), and seprafilm and interceed TC7 have been tested

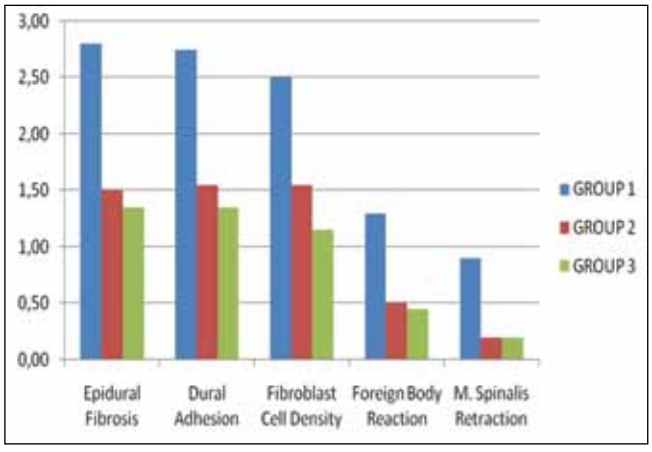

Figure 1: Comparative graphics of mean values. The values in group 2 (topical citicoline group) and group 3 (systemic citicoline group) were significantly lower than those in group 1 (0,9% saline group). Significance levels between group 1 and 2 with regard to epidural fibrosis, dural adhesion, fibroblast cell density, foreign body reaction and medulla spinalis retraction were p<0,001, p<0,001, p<0,001, p<0,021, and p<0,043, respectively. Significance levels between group 1 and 3 with regard to epidural fibrosis, dural adhesion, fibroblast cell density, foreign body reaction and medulla spinalis retraction were p<0,001, p<0,001, p<0,001, p<0,013, and p<0,033 respectively. There were no significant differences between group 2 and 3 in terms of the measured parameters (p>0,05).

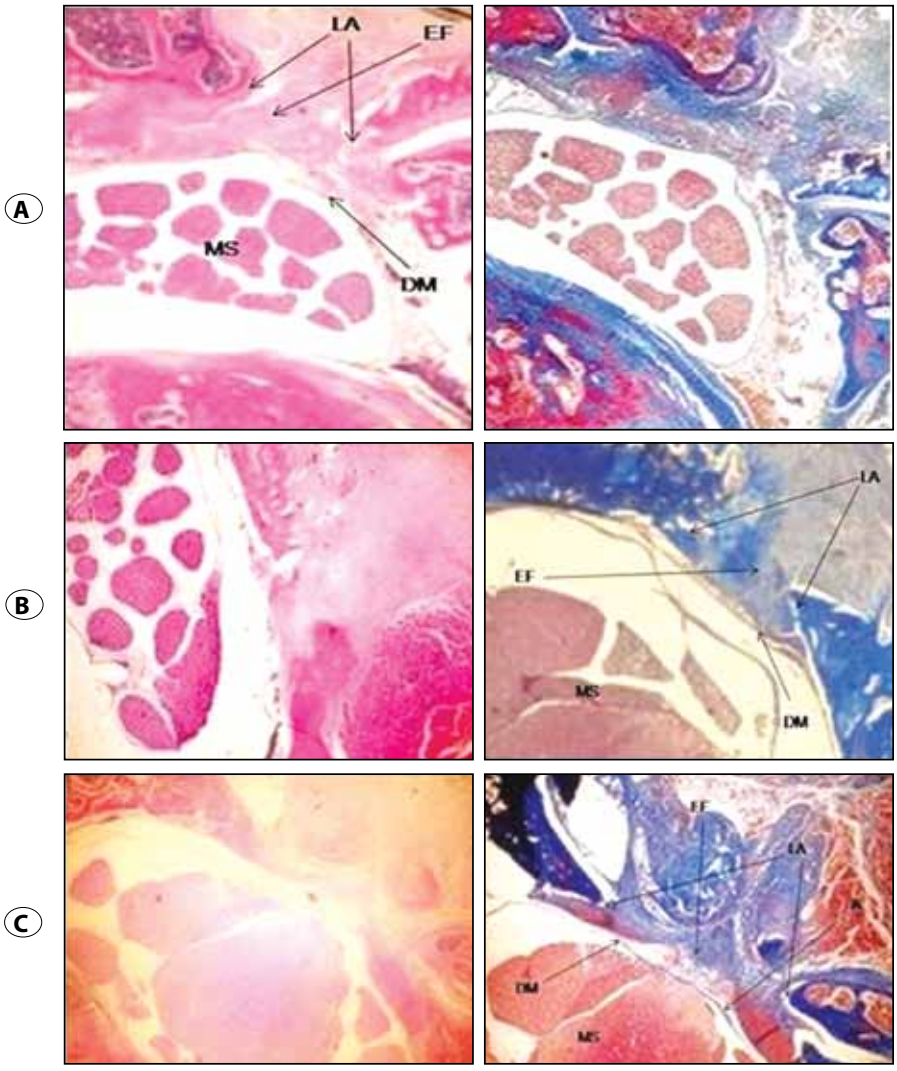

Figure 2: Representative images of Grade 3 **(A)**, Grade 2 **(B)** and Grade 1 **(C)** epidural fibrosis. Images on the left panel were obtained by Hemotoxylin-eosine (HE) staining and those on the right panel were obtained by Masson's trichrome (MT) staining. **EF:** Epidural fibrosis, **LA:** laminotomy area, **DM:** Dura mater, **MS:** Medulla spinalis.

for preventing epidural fibrosis, however, none of them have entered clinical use (8, 16, 18, 19, 21, 23, 29, 42, 46). Therefore, new studies are required in order to discover new materials or chemicals that could prevent epidural fibrosis.

CDP-choline (cytidine 5'- diphosphocholine), is an endogenous intermediate in the biosynthesis of cell membrane phospholipids and exogenously-administered CDP-choline is referred to as citicoline (53). Citicoline and its metabolite choline serve as substrates for the synthesis of phosphatidylcholine, a primary component of neuronal membrane (2, 17, 53). Grouped with the B vitamins, choline is a trimethylated nitrogenous base that enters three major metabolic pathways: (1) phospholipid synthesis via phosphorylcholine; (2) acetylcholine synthesis; and (3) oxidation to betaine, which serves as a methyl donor (17).

Citicoline has been shown to exhibit beneficial effects in several neurological conditions. For example, citicoline improves neurological outcomes and reduces the volume of ischemic injury in patients with stroke (17, 41). In addition, citicoline reduces infarct size and decreases mortality in hypotensive rats with subarachnoid hemorrhage and basilar artery occlusion (4).

The observed pathologies with regard to epidural fibrosis in the present rat laminotomy and discectomy model suggest the onset of inflammatory processes after the surgery. Hence, treatments targeting the inflammatory response might confer benefit in lumbar disc surgery. We found reduced scar formation accompanied by decreased dural adhesion, fibroblast cell density, foreign body reaction and medulla spinalis retraction following either local or systemic administration of citicoline in our study. The mechanism(s) whereby citicoline exhibits such effects are not yet known. However, evidence for possible citicoline mechanism of action has been provided in previous studies. For example, citicoline was reported to decrease phospholipase A2 stimulation thereby leading to blockade of generation of inflammatory prostaglandins and thromboxanes (1).

Although these anti-inflammatory effects seem to have resulted from direct action of citicoline, it is also possible that choline metabolized from citicoline might have contributed

Table IV: Grading Scores of all Parameters in Three Groups

Rat No	Density of Epidural fib.			Dural Adhesion			Fibroblast Cell Density			Foreign Body Reaction			Medulla Spinalis Retraction		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
1	3	2	2	3	2	2	3	2	2	3	2	0	0	0	3
2	3	2	1	3	2	1	3	1	1	3	0	0	3	1	0
3	3	2	2	2	2	2	3	1	1	2	0	0	1	0	0
4	3	1	1	2	1	2	3	1	1	3	0	0	0	0	0
5	2	1	1	2	1	1	3	1	1	1	1	0	2	0	0
6	3	2	1	3	2	1	3	2	1	0	0	0	2	0	0
7	3	2	1	3	2	1	3	2	1	0	1	1	0	0	1
8	3	2	1	3	1	1	2	3	2	1	0	0	0	0	0
9	3	1	2	3	2	1	1	2	1	1	1	0	0	0	0
10	3	2	1	3	2	1	3	2	1	2	0	0	1	0	0
11	3	2	2	3	2	1	2	2	1	0	0	0	2	0	0
12	3	1	1	3	1	2	3	1	1	2	0	1	1	2	0
13	3	1	2	3	1	2	2	1	1	2	0	1	1	0	0
14	3	1	1	3	2	1	3	2	1	2	1	1	0	0	0
15	2	1	2	3	1	1	3	2	1	1	2	1	0	0	0
16	3	2	1	2	1	2	1	1	2	2	0	1	0	0	0
17	3	1	1	3	2	2	3	1	1	1	0	1	0	1	0
18	3	2	1	3	1	1	3	2	1	0	1	1	2	0	0
19	2	1	2	3	2	1	2	1	1	0	0	0	3	0	0
20	2	1	1	2	1	1	1	1	1	0	1	1	0	0	0

to the observed therapeutic effects. Recent studies demonstrated anti-inflammatory properties of cholinergic activation and the term "cholinergic antiinflammatory pathway" has been proposed (50). CDP-choline is a choline donor since part of exogenous CDP-choline is metabolized to choline (14, 54). While choline deficiency results in increased levels of proinflammatory cytokines such as TNF-α (25), parenteral choline administration attenuates endotoxin-induced multiple organ injury and the elevation in circulating TNF-→ levels (27). Since CDP-choline is a source of choline and its administration leads to initiation of responses in cholinergic nature (15, 28), it could be speculated that one mechanism by which CDP-choline ameliorates the severity of scar formation after lumbar disc surgery might be the action of choline that was released by citicoline breakdown on inflammatory processes. This hypothesis is further supported by evidence from a previous study in which we demonstrated beneficial effects of both citicoline and choline on reducing postoperative scarring in a rat model of peripheral nerve injury (6). In addition, reversal of mechanical hyperalgesia in carrageenan-induced inflammatory pain model after citicoline administration (7) was mediated in part by supraspinal α7-selective nicotinic ACh receptors which utilizes choline as a selective agonist (38). Therefore, our present observations could have resulted from possible blockade of the inflammatory phase of scar formation by citicoline or its metabolite choline. These possible mechanisms are now being tested in our laboratory.

CONCLUSIONS

To the best of our knowledge, this is the first experimental study demonstrating prevention of epidural fibrosis by citicoline in a rat model of laminotomy and discectomy. Our present data show that citicoline effectively prevents epidural fibrosis by either local or systemic administration. Future studies are warranted in order to determine citicoline's mechanisms of action on epidural fibrosis and to test its clinical efficiency.

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