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Effects of Topical Cyclosporin A Application on Preventing Epineural Scar Formation in Rats: Experimental Study

Sıçanlarda Topikal Siklosporin A Uygulamasının Epinöral Skar Oluşumunu Önlemedeki Etkileri

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ABSTRACT

The aim of this study is to evaluate macroscopic, histopathologic and immunohistochemical effects of topical cyclosporin administration on prevention of epineural scar formation in rats.
This experimental study was performed in two groups, each consisting of ten rats. Sciatic nerve was opened bilaterally. Tibial and peroneal components were set apart with blunt dissection. Abrasion injury was achieved by repetitive rubbing over biceps femoris muscle. In the control group saline sucked cotton peds were administered over opened sciatic nerve region bilaterally, whereas cyclosporin sucked peds were administered in the second group for five minutes duration. Eight weeks after surgery both groups were sacrificed and nerve complexes were evaluated microscopically, histopathologically and immunohistochemically.
No side effects were observed after 5 minutes single dose topical cyclosporine administration in our study. Cutaneous, muscular and deep fascial repairment were almost completed according to Petersen's numerical grading system ($p<0.05$). Nerve adherence was significantly decreased ($p<0.001$) in the ones treated with cyclosporin than the control group. FGF expression was demonstrated and individual evaluations of the control and the study groups immunhistochemically. The ratio of fibroblast/fibrosit number showed that both groups results were parallel. Single dose topical cyclosporin administration is shown to be successfull in preventing epineural scar formation after peripheric nerve neurolysis.

Keywords: Epineural scarring, Cyclosporin A, Pheripherial Nerve Surgery

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ÖZ

Bu çalışmanın amacı, sıçanlarda epinöral skar oluşumunun önlenmesinde topikal siklosporin uygulamasının makroskopik, histopatolojik ve immünohistokimyasal etkilerini değerlendirmektir.

Bu deneysel çalışma, her biri on rattan oluşan iki grupta gerçekleştirildi. Siyatik sinir iki taraflı açıldı. Tibial ve peroneal komponentler künt diseksiyonla ayrıldı. Biceps femoris kası üzerine tekrarlayan sürtmelerle abrazyon hasarı oluşturuldu. Kontrol grubuna açılan siyatik sinir bölgesine bilateral salin emdirilmiş pamuklu ped, ikinci gruba 5 dakika süreli siklosporin emdirilmiş ped uygulandı. Ameliyattan sekiz hafta sonra her iki grup da sakrifiye edildi ve sinir kompleksleri mikroskobik, histopatolojik ve immünohistokimyasal olarak değerlendirildi.

Çalışmamızda 5 dakikalık tek doz topikal siklosporin uygulamasından sonra herhangi bir yan etki gözlenmedi. Petersen'in sayısal derecelendirme sistemine göre deri, kas ve derin fasiyal onarım neredeyse tamamlandı ($p<0.05$). Siklosporin ile tedavi edilenlerde sinir adezyonu kontrol grubuna göre anlamlı olarak azaldı ($p<0.001$). FGF ekspresyonu gösterildi ve kontrol ve çalışma gruplarının bireysel değerlendirmeleri immünhistokimyasal olarak yapıldı. Fibroblast/fibrosit sayısının oranı her iki grubun sonuçlarının paralel olduğunu gösterdi. Tek doz topikal siklosporin uygulamasının, periferik sinir nörolizinden sonra epinöral skar oluşumunu önlemede başarılı olduğu gösterilmiştir.

Anahtar Kelimeler: Epinöral skar, Siklosporin A, Periferik Sinir Cerrahisi

INTRODUCTION

Although new surgical techniques have been developed, epineural scar formation following peripheral nerve surgery is still one of the major problems on the postoperative period of clinical outcome. Especially for the recurring surgical requirement, epineural scar formation plays an important role on the surgical success.

Epineural scarring is one of the important factors affecting postoperative clinical results and success of surgical procedure. Epineural scarring restricts the nerve mobility by tethering the peripherial nerves during the limb movement. Severe and prolonged tethering of the nerves may cause ischemia and further nerve injury. In the clinical course tethering of the nerves results not only pain but also sensorial and motor deficits due to compression of the nerves [1-4].

Preventing from or reduction in epineural scarring increases the peripherial nevre surgery sucsess, decreases the complications and facilitates the secondary operations [5,27].

Cyclosporin A, a wellknown nonpolar cyclic oligopeptid immunsupressif agent widely used in organ transplantation and in the field of ophthalmology for preventing postoperative fibrosis.

Studies reveal that cyclosporin A prevents fibroblastic proliferation and decrease the fibroblast collagen synthesis [19-21,23]. Intraoperative topical cyclosporin administration which may prevent epineural fibrosis was examined by gross anatomically, histopathologically and immunohistochemically

MATERIALS AND METHODS

Twenty adult male Wistar rats weighing 250–300 g were housed in a temperature- and humidity-controlled room (22±3°C and 67±7%, respectively) in which a 12- to 24-h light–dark cycle was maintained. The animals were fed standard rat chow and tap water ad libitum. Ethical approval was granted by the University Ethics Committee.

Surgical Procedure

General anesthesia was established by ether inhalation. Sciatic nerve was opened bilaterally set apart from the neighboring structures by sterile technique. Tibial and peroneal components were separated via blunt dissection toward the sciatic foramen. Abrasion injury was achieved by repetitive rubbing of the nylon tooth-brush over biceps femoris muscle. Meanwhile the nerves were kept retracted carefully. Saline sucked peds were administered around sciatic nerves bilaterally of the control group and 0.3ml of 50mg/ml cyclosporin-A sucked peds were

administered around sciatic nerves bilaterally of the control group for five minutes duration. Afterwards fascia was closed with 3/0 vicryl and skin was closed with stapler.

Evaluations

Evaluations were performed macroscopically, histopathologically and immunohistochemically respectively.

Postoperative observations

After the operation, rats were examined weekly for healing characteristics of the wound site and for sciatic nerve functions, including abnormal foot posture, toe spreading, foot dorsoflexion, and plantar flexion.

Gross anatomical evaluations

Blind surgical dissection was performed to the neurolysis sites of half the rats in the Cyclosporine treated group and the untreated control group under deep ether anesthesia after 8 weeks surgery. Perineural adhesions during anatomical dissection were evaluated concerning the numerical grading scheme described by Petersen et al.[4], (Table 1).

Histolopathologic and Immunohistologic study

The remaining half of the rats in the cyclosporine treated group and the untreated control group were used for histological evaluation after 8 weeks surgery. All animals were sacrificed by deep ether anesthesia and perfused-fixed with 0.9% saline followed by 2% paraformaldehyde. The entire sciatic nerve and surrounding scar tissue were removed en bloc and immersed in 10% neutral buffered formalin overnight. Tissues were embedded in paraffin and cross-sectioned at 5 µm. Sections were stained with hematoxylin and eosin. Connective tissue was evaluated using Masson trichrome stain. The thicknesses of scar and nerve tissue were measured under light microscopy (Olympus BX51, Tokyo, Japan) using an ocular micrometer (Olympus), and the scar tissue formation index was obtained by dividing the value of the thickness of the scar tissue by the value of the thickness of the nerve tissue [6,27].

Under 40× magnification, the fibroblasts/fibrocytes were counted for four different quadrants around the epineurium for each nerve, and the mean number of fibroblasts/fibrocytes was calculated. Each specimen was graded according to the following scale, which has been reported by some authors [7,27]. Grade 1 less than 100 fibroblasts, Grade 2 100–150 fibroblasts, Grade 3 more than 150 fibroblasts.

The sections were waited in etui at 56°C for a night, prepared with xylol, absolute and normal alcohol, boiled in sitrate buffer for 30minutes and undergone hydrogen-peroxide for immunohistochemical study. Antibody (fibroblast growth factor) (Santa Cruz Biotechnology 147 sc-79) primary antibody and chromogen were performed. Adverse staining with Mayer hematoxilene was made. Fibrosis of perineurium was evaulated under 40x and 100x light microscope. Staining degrees of epineural area with FGF-2 were evaluated via semi-quantitative method by two investigators, who were blind to the groups.

A modified scale smiliar to the litreture was used for statistical evaluation [28]. Staining for FGF-2 (increasing of the brown dansity of the epineural area) was rated as normal (+), increased (++) and extremely increased (+++).

Statistical Analysis

Statistical analysis was performed with software SPSS 10 program under windows XP. Independent t-test is used for the significance among the groups and dependent t-test is used for

comparing right and left leg sciatic nerve for each rat. $p<0.05$ is accepted as significant.

RESULTS

Clinical Results

There was no statistical significance between characterization of wound area healing and the neurological function of the leg among the rats treated with cyclosporine and the non-treated ones ($p>0.05$).

Anatomical Results

Cutaneous sutures were removed at the end of the 8th week and the original incision areas were reopened and evaluated carefully. Enflammautory reaction findings were not observed. The epineural adhesions surrounding the nerves were significantly less than the control group (Fig 1 A, B). Cutaneous, muscular and deep fascia closure according to Petersen's numerical grading scale was almost completed ($p>0.05$). Nerve adhesions in the cyclosporine treated group was significantly decreased compared to the control group ($p<0.001$). Gross anatomical evaluation results are summarized in Table 1.

Histopathological Results

Dense epineural connective tissue, like a thick band surrounding the nerves in the saline administered control group and the tissue surrounding the nerves in the control group were demonstrated and compared with 40X magnifing with the light microscope (Fig 2 A, B).

Statistical significance was not determined among the right and left siatic nerves in both group by qualitative analysis of the connective tissue surrounding the nerves (scar tissue index), however there was a statistically significance between the cyclosporine treated group and the control group ($p<0.01$) (Table 2,3).

The ratio of fibroblast per fibrosit number were found to be significantly increased in the control group than the cyclosporine treated group ($p<0.001$) (Table 4).

Immunohistochemical Results

In immunohistochemical studies, after staining for FGF-2, FGF expression was demonstrated at 40X and 100X magnifing with light microscope. As mentioned in the literature[28] and due to our

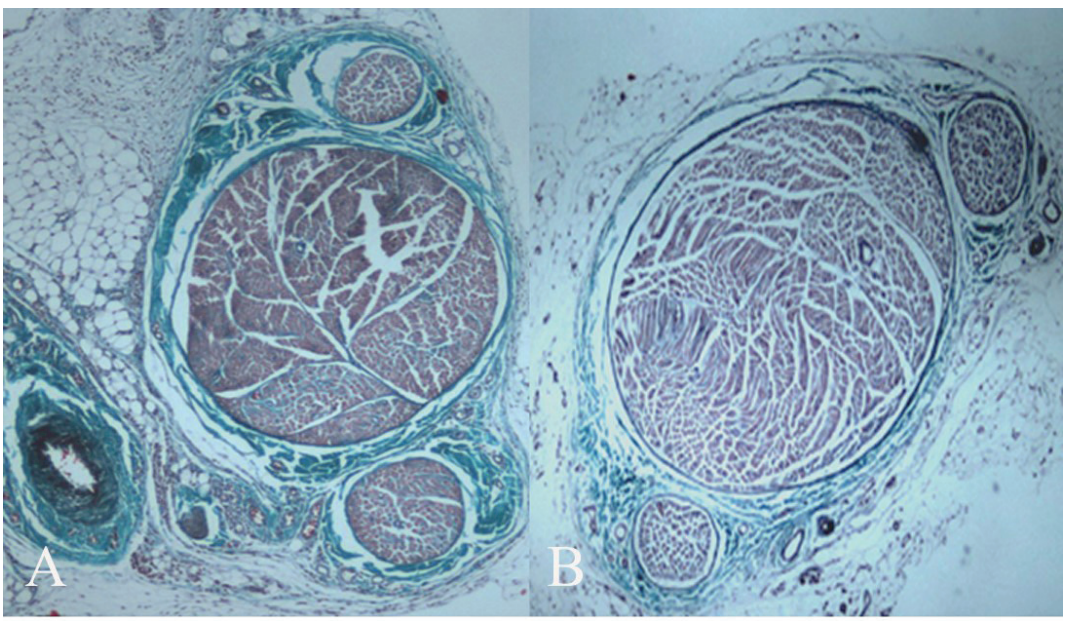

Figure 1. The epineural adhesions surrounding the nerves. A: Control group, B: Study group.

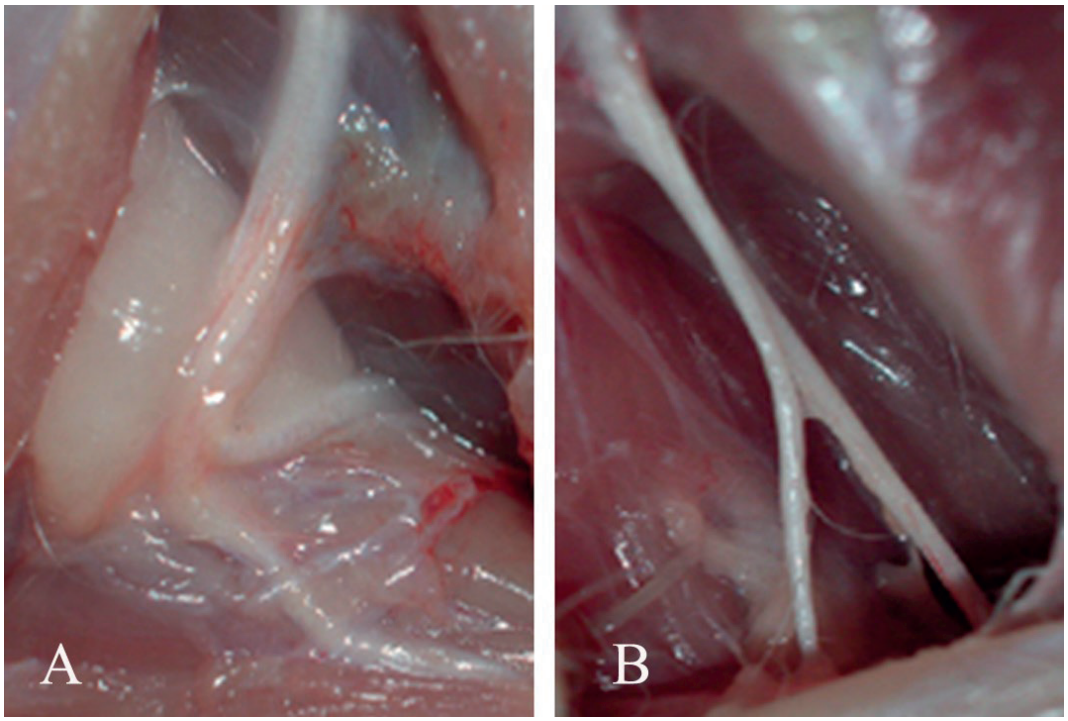

Figure 2. Images under 40X magnifying light microscope of epineural tissues in the (A) control and (B) study group respectively.

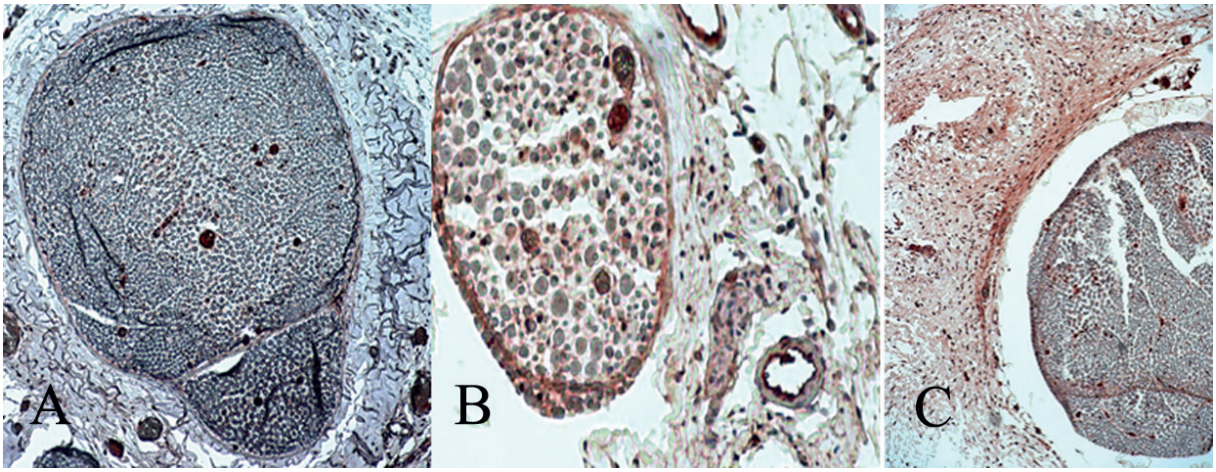

Figure 3. FGF Staining. The brown color of the epineural area increases as the (+) increase. A: 1(+) FGF expression, B: 2(++) FGF expression, C: (+++) FGF expression.

modified scale, as (+) increase, increasing of the brown dansity of the epineural area was observed (Fig 3 A, B, C). Both investigators' blind evaluation results were evaluated statistically. Independent t test results revealed significance among the study group and the control group ($p<0.001$) (Table 5).

Individual evaluations of the control and the study groups immunhistochemically, the ratio of fibroblast /fibrosit number showed that both groups results were parallel (Table 6).

Table 1. Peterson grading and gross anatomical evaluation results. Independent t-test results of comparing with control group (p<0.001).

Nerve No	Skin Closure		Muscular fascia		Nerve tissue		Separateness	
	control	CsA	control	CsA	control	CsA	control	CsA
1	1	1	1	2	2	1	2	1
2	1	1	1	1	2	2	2	1
3	1	1	2	1	2	1	3	1
4	1	1	2	1	2	1	2	1
5	1	1	1	2	3	2	2	2
6	1	1	1	1	3	1	3	1
7	1	1	1	1	3	2	2	1
8	1	1	1	1	2	1	3	1
9	1	1	1	1	3	1	2	1
10	1	1	1	2	2	2	3	1
Mean±SD	1,00±0,00	1,00±0,00	1,20±0,42	1,30±0,48	2,40±0,52	1,40±0,52*	2,40±0,52	1,10±0,32*

Table 2. Comparison scar tissue index of each rat's right and left sciatic nerve and dependent t test results. Dependent t test results revealed that there was no statistical significance between right and left legs of control group (p=0,608) or the study group (p=0,800).

Nerve no R-L	Group 1		Group 2	
	R	L	R	L
1-6	0,139	0,164	0,081	0,053
2-7	0,189	0,116	0,046	0,055
3-8	0,112	0,150	0,047	0,052
4-9	0,142	0,131	0,043	0,047
5-10	0,157	0,122	0,028	0,049
Mean±SD	0,1478±0,03	0,13660±0,02	0,0490±0,02	0,0512±0,003

Table 3. Comparison of scar tissue formation among control group and the study group and the results of independent t-test. Independent t-test results of comparing with control group (p<0.001).

Nerve no	Control	CsA
1	0,139	0,081
2	0,189	0,046
3	0,112	0,047
4	0,142	0,043
5	0,157	0,028
6	0,164	0,053
7	0,116	0,055
8	0,150	0,052
9	0,131	0,047
10	0,122	0,049
Mean±SD	0,14220±0,02	0,05010±0,01*

Table 4. Comparing Fibroblast/fibrosit number. Independent t-test results of comparing with control group (p<0.001).

Nerve No	Control	CsA
1	3	1
2	3	2
3	3	1
4	1	2
5	2	1
6	2	1
7	3	2
8	3	2
9	2	1
10	3	1
Mean±SD	2,50±0,7	1,40±0,5*

Table 5. Staining for Fibroblast growth factor. Independent t-test results of comparing with control group (p<0.001).

Nerve No	Control	CsA
1	3	2
2	2	1
3	3	2
4	2	2
5	2	1
6	2	1
7	3	2
8	3	1
9	2	2
10	3	1
Mean±SD	2,50±0,5	1,50±0,5*

In recent years many agents including human amniotic fluid, fibrine glue, cis-hydroxyprolin, antitransforming growth factor-ß antibody, aprotinin, ADCON_T/N, mitomycine and low dose radition therapy were tried on the prevention of epineural scar formation [14-18,27]. Moreover large clinical series that reports the usefullness of these materials are not present.

Cyclosporin A, a chemotherapoetic agent that is used especially in ophtalmatology, is shown to be effective for prevention of postoperative fibrosis.

Table 6. Comparison fibroblast/fibrosit number of histopathologic evaluation and immunohistochemical results among both control and study groups.

Nerve No	Fibroblast number		Degree of staining for Fibroblast growth factor	
	Control	CsA	Control	CsA
1	3	1	3	2
2	3	2	2	1
3	3	1	3	2
4	1	2	2	2
5	2	1	2	1
6	2	1	2	1
7	3	2	3	2
8	3	2	3	1
9	2	1	2	2
10	3	1	3	1
Mean ±SD	2,50±0,7	1,40±0,5	2,50±0,5	1,50±0,5

DISCUSSION

Prevention or decresing of epineural scar formation increases the success of peripheric nerve surgery, fasciliates the following surgical procedure and decreases the complication rates [8,9].

Many surgical techniques were developed to decrease or prevent the amount and frequency of epineural scar formation, secondary neurolysis, microsurgical techniques, endoscopic techniques, nerve transposition, dermofascial fat grafts, vein packing and muscular flaps [10-14]. However performing of very special techniques can not prevent postoperative adhesion formation.

On acute rejection of transplated organs, intragraft fibroblasts increases hyaluronan production. Cyclosporine, decreases this production about 50% [16].

Literature on cyclosporine proposes that it affects as decreasing the enflammatory cells by incresing the number of mucine producing Goblet cells [22,24-26]. A study has shown that cyclosporine directly affects the natural duration of fibroblasts and increases apoptozis on fibrotic tissues clinically [19]. Furthermore some studies have shown the relation between cyclosporine A effect on pericardial enflammation on complete and stable remission [20]. It's shown that in contrast to chemotreapotic agents like azotioprine and

cyclophosphamides, cyclosporin does not destroy the immun effectors, but inhibites the activation and proliferation of T cells especially T helpers. Cyclosporine's molecular mechanism is shown to be associated with IL-2 synthezis [20,24].

Invivo and invitro studies revealed that cyclosporin A prevents fibroblastic proliferation and decreases fibroblast collagen synthesis [19-22].

Our results demonstrate that single dose topical cyclosporine administration was significantly effective on prevention of epineural scar formation. Skin closure were completed on both groups. In the study group, there was no faillure of siatic nerve and surrounding tissues were significantly decreased in the study group, also nerve could be easily put apart from surrounding muscular gap. Significant scar formation was observed in the control group. Nerve adhesion scores were found to be significantly low in the cyclosporine treated group compared with the control group.

Results of quantitative histological and immunological evalutaion of the scar tissue supported the gross evaluation results. Scar tissues thickness were significatly higher in the control group compared with the cyclosporine treated group. Fibroblast/fibrocite number ratio was found to be significantly lower in the study group. After painting with FGF, FGF expression was significantly incressed in the study group compared with the control group.

Local and systemic administration of cyclosporine may result with dose related complication. However low dose and short administration time decreases these complications. We did not observe any side effect after single dose topical administration for 5 minutes.

CONCLUSION

In peripheric nerve surgery epineural scar formation plays an important role on the postoperative clinical results and evaluation of surgical success. Single dose topical cyclosporine administration is shown to be successfull in preventing epineural scar formation after peripheric nerve neuronalysis. Local advers effect was not seen. However further studies should be performed on the neccessary concentration and exposure time of the long term security.

Ethical Approval: This study was approved by the Kocaeli University Ethics Committee (No: 97 / 04.05.2005).
Conflict of interest: There is no conflict of interest in our study.
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REFERENCES

1. Dam-Hieu P, Lacroix C, Said G, Devanz P, Liu S, Tadie M. Reduction of postoperative perineural adhesions by Hyaloglide gel: an experimental study in the rat sciatic nerve. Neurosurgery. 2005; 56(2 Suppl): 425-433. https://doi.org/10.1227/01.NEU.0000156845.41626.E9
2. Shaw Wilgis EF: Clinical aspect of nerve gliding in the upper extremity, in Hunter JM, Schneider LH, Mackin EJ (eds): Tendon and Nerve Surgery in the Hand. St. Louis, C.V. Mosby, Inc., 1997: 121-124.
3. Adzick NS, Lorenz HP. Cells, matrix, growth factors, and the surgeon. The biology of scarless fetal wound repair. Review. Ann Surg. 1994; 220(1): 10-18. https://doi.org/10.1097/00000658-199407000-00003

4. Petersen J, Russell L, Andrus K, MacKinnon M, Silver J, Kliot M. (1996) Reduction of extraneural scarring by ADCON-T/Nafter surgical intervention. Neurosurgery 38:976-984. https://doi.org/10.1097/00006123-199605000-00025
5. Smit X, van Neck JW, Afoke A, Hovius SE. Reduction of neural adhesions by biodegradable autocrosslinked hyaluronic acid gel after injury of peripheral nerves: an experimental study. J Neurosurg. 2004; 101(4): 648-652. https://doi.org/10.3171/jns.2004.101.4.0648
6. Ozgenel GY, Filiz G. Effects of human amniotic fluid on peripheral nerve scarring and regeneration in rats. J Neurosurg. 2003; 98(2): 371-377. https://doi.org/10.3171/jns.2003.98.2.0371
7. Hinton JL Jr,Warejcka DJ, Mei Y, McLendon RE, Laurencin C,Lucas PA, Robinson JS Jr (1995) Inhibition of epidural scar formation after lumbar laminectomy in the rat. Spine 20:564-570. https://doi.org/10.1097/00007632-199503010-00011
8. Wilgis EF, Murphy R. The significance of longitudinal excursion in peripheral nerves. Hand Clin 1986; 2: 761-766. https://doi.org/10.1016/S0749-0712(21)00622-3
9. Nachemson AK, Lundborg G, Myrhage R, Rank F. Nerve regeneration and pharmacological suppression of the scar reaction at the suture site. An experimental study on the effect of estrogen-progesterone, methylprednisolone-acetate and cis-hydroxyproline in rat sciatic nerve. Scand J Plast Reconstr Surg.1985; 19: 255-260. https://doi.org/10.3109/02844318509074512
10. Mastronardi L, Pappagallo M, Puzzilli F, Tatta C. Efficacy of the morphine-Adcon-L compound in the management of postoperative pain after lumbar microdiscectomy. Neurosurgery. 2002; 50(3): 518-525. https://doi.org/10.1227/00006123-200203000-00017
11. Akeson WH, Massie JB, Huang B, Giurea A, Sah R, Garfin SR, et al. Topical high-molecular-weight hyaluronan and a roofing barrier sheet equally inhibit postlaminectomy fibrosis. Spine J. 2005; 5(2): 180-190. https://doi.org/10.1016/j.spinee.2004.06.019
12. Gorgulu A, Uzal C, Doganay L, Imer M, Eliuz K, Cobanoglu S. The effect of low-dose external beam radiation on extraneural scarring after peripheral nerve surgery in rats. Neurosurgery. 2003; 53(6): 1389-1396. https://doi.org/10.1227/01.NEU.0000093827.05319.E5
13. de Tribolet N, Porchet F, Lutz TW, Gratzl O, Brotchi J, van Alphen HA, et al. Clinical assessment of a novel antiadhesion barrier gel: prospective, randomized, multicenter, clinical trial of ADCON-L to inhibit postoperative peridural fibrosis and related symptoms after lumbar discectomy. Am J Orthop. 1998; 27(2): 111-120.
14. Turgut M, Uysal A, Pehlivan M, Oktem G, Yurtseven ME. Assessment of effects of pinealectomy and exogenous melatonin administration on rat sciatic nerve suture repair: an electrophysiological, electron microscopic, and immunohistochemical study. Acta Neurochir(Wien). 2005; 147(1): 67-77. https://doi.org/10.1007/s00701-004-0426-x
15. Einhaus SL, Robertson JT, Dohan FC Jr, Wujek JR, Ahmad S. Reduction of peridural fibrosis after lumbar laminotomy and discectomy in dogs by a resorbable gel (ADCON-L). Spine. 1997: 22(13); 1440-1446. https://doi.org/10.1097/00007632-199707010-00003
16. Menovsky T, Beek JF. Laser, fibrin glue, or suture repair of peripheral nerves: a comparative functional, histological, and morphometric study in the rat sciatic nerve. J Neurosurg. 2001 Oct; 95(4):694-699. https://doi.org/10.3171/jns.2001.95.4.0694
17. Isla A, Martinez JR, Perez-Lopez C, Perez Conde C, Morales C, Budke M. A reservable antiadhesion barrier gel reduces the perineural adhesions in rats after anastomosis. J Neurosurg Sci. 2003; 47(4): 195-200.
18. Palatinsky EA, Maier KH, Touhalisky DK, Mock JL, Hingson MT, Coker GT. ADCON-T/N reduces in vivo perineural adhesions in a rat sciatic nerve reoperation model. J Hand Surg [Br]. 1997; 22(3): 331-335. https://doi.org/10.1016/S0266-7681(97)80397-X
19. Leonardi A, DeFranchis G, Fregona IA, Violato D, Plebani M, Secchi AG. Effects of cyclosporin A on human conjunctival fibroblasts. Arch Ophthalmol. 2001; 119: 1512-1517. https://doi.org/10.1001/archopht.119.10.1512
20. Lessio S, Laveder F, Marcolongo R, Rigoli A, Tona. Cyclosporin A in the treatment of idiopathic recurrent pericarditis: a case report. J Clin Basic Cardiol 1999; 2: 130-131.
21. Furue M, Gaspari AA, Katz SI. The effect of cyclosporin A on epidermal cells. II. Cyclosporin A inhibits proliferation of normal and transformed keratinocytes. J Invest Dermatol. 1988 ; 90(6): 796-800. https://doi.org/10.1111/1523-1747.ep12462009
22. Kunert KS, Tisdale AS, Stern ME, Smith JA, Gipson IK Analysis of topical cyclosporine treatment of patients with dry eye syndrome: effect on conjunctival lymphocytes. Arch Ophthalmol. 2000; 118: 1489-1496. https://doi.org/10.1001/archopht.118.11.1489
23. Yamaguchi M, Naruishi K, Yamada-Naruishi H, Omori K, Nishimura F, Takashiba S. Long-term cyclosporin A exposure suppresses cathepsin-B and -L activity in gingival fibroblasts. J Periodontal Research. 2004; 39(5): 320. https://doi.org/10.1111/j.1600-0765.2004.00746.x

24. Kunert KS, Tisdale AS, Gipson IK. Goblet cell numbers and epithelial proliferation in the conjunctiva of patients with dry eye syndrome treated with cyclosporine. Arch Ophthalmol. 2002; 120(3): 330-337. https://doi.org/10.1001/archopht.120.3.330
25. Cross WD, Lay LF Jr, Walt JG, Kozma CM. Clinical and economic implications of topical cyclosporin A for the treatment of dry eye. Manag Care Interface. 2002; 15(9): 44-49.
26. Murphy LL, Hughes CC. Endothelial cells stimulate T cell NFAT nuclear translocation in the presence of cyclosporin A: involvement of the wnt/glycogen synthase kinase-3 beta pathway. J Immunol. 2002; 169(7): 3717-3725. https://doi.org/10.4049/jimmunol.169.7.3717
27. Ilbay K, Etus V, Yildiz K, Ilbay G, Ceylan S. Topical application of mitomycin C prevents epineural scar formation in rats. Neurosurg Rev. 2005; 28(2): 148-153. https://doi.org/10.1007/s10143-004-0370-5
28. Strutz F, Zeisberg M, Hemmerlein B, Sattler B, Hummel K, Becker V, et al. Basic fibroblast growth factor expression is increased in human renal fibrogenesis and may mediate autocrine, fibroblast proliferation. Kidney Int. 2000; 57(4): 1521-1538. https://doi.org/10.1046/j.1523-1755.2000.00997.x