Original Araştırma

Inhibition of Epidural Fibrosis After Laminectomy Using Topical Aminoguanidine in A Rat Model

Rat Modelinde Laminektomi Sonrası Epidural Fibrozisin Topikal Aminoguanidin Kullanılarak İnhibisyonu

M. Arif Aladağ

Inönü University, Medical School, Departments of Neurosurger, Malatya, Turkey

Özet

Amaç: Bu deneysel çalışmada, laminektomi sonrası epidural fibrozisin indüklenebilir nitrik oksit sentaz (iNOS) inhibitörü olan aminoguanidin topikal uygulanması ile önlenmesini araştırdık.

Gereç ve Yöntem: Bu çalışmada her biri 10 rat içeren 3 grup oluşturuldu. kullandık. Tüm ratlarda lomber laminektomi yapıldı. Kontrol grubu (grup1) epidural mesafeye herhangi bir malzeme yerleştirilmeden kapatıldı. Spongostan ve spongostan artı aminoguanidin gruplarında epidural mesafeye, kapatmadan önce sırasıyla salin (0.9 % NAC) emdirilmiş spongostan ve aminoguanidin (50 mg/kg B. W.) emdirilmiş spongostan yerleştirildi. Bu hayvanların hepsi operasyondan sonraki 14. günde sakrifiye edildi. Her bir ratda histolojik olarak ektradural fibrozis ve kompresyon değerlendirildi.

Sonuçlar: Spongostan artı aminoguanidin grubunda fibröz proliferasyon veya kompresyon yokken, kontrol ve spongostan gruplarında, sırasıyla belirgin ve orta derecede fibrozis ve kompresyon mevcuttu.

Sonuç: Aminoguanidinin, laminektomi sonrası epidural fibrozis ve kompresyonun önlenmesinde ideal bir inhibitör olabileceği gösterildi.

Anahtar Kelimeler: Rat, laminektomi, epidural fibrozis, aminoguanidin, spongostan.

Abstract

Aim: In this experimental study, we investigated prevention of epidural fibrosis following laminectomy with topical application of aminoguanidine, a selective inhibitor of inducible nitric oxide synthase (iNOS).

Materials and Methods: We used 3 groups each containing 10 male wistar rats. All rats were made laminectomy in lumbar spine. The control group was closed without placing any material on the epidural space. In the spongostan and spongostan plus aminoguanidine, groups the saline (0.9 % NaCl) soaked spongastan and aminoguanidine (50 mg/kg B.W.) soaked spongostan were placed epidurally respectively before closing. All of these animals were sacrificied on the 14th days after operation. The extradural fibrosis and compression in every animal were evaluated by histological examinations.

Results: While there was not any fibrous proliferation or compression in the group of spongostan plus aminoguanidine group. There were obvious and moderate extradural fibrosis and compression in the control and spongostan groups.

Conclusion: It was suggested that aminoguanidine may be an ideal inhibitor in the prevention of epidural fibrosis and compression following laminectomy.

Keywords: Rat, laminectomy, epidural fibrosis, aminoguanidine, spongostan.

Introduction

Epidural fibrosis is a natural consequence of the normal postlaminectomy and/or discectomy wound healing. But, this can lead to symptoms by compressing or tethering to the dural sac and/or to the nerve roots. In spite of decreasing rate of scar formation after lumbar or lumbosacral laminectomy and/or discectomy with advances in surgical techniques, it has often been reported that epidural fibrosis still is the one of common cause of postoperative low back and/or radicular pain, so-called the failed back surgery syndrome (1-14).

Although many synthetic and biological materials to prevent postlaminectomy scarring or fibrosis have been used experimentally and clinically, there is not universal consent about these methods (15-27). On the other hand, the materials used to prevent postlaminectomy scarring, may also cause fibrosis themselves, because of foreign body reaction to them. (26-28).

A short-lived free radical nitric oxide plays an important role in the healing of various types of wounds. Many experimental studies have shown that nitric oxide (NO) produced by inducible nitric oxide synthases (iNOS) in macrophages and other cells in wound in earlier stage of wound healing (6 to 24 hours after injury), and, iNOS expression is restricted to the period of the initial 24 to 72 hours after injury. It was revealed that NO produced by iNOS cause the collagen accumulation in earlier stage in acute wounds. NO production from iNOS and collagen accumulation decrease and almost finish in later stages (29-36). It also was shown

that inhibition of nitric oxide synthasis in wounds impaired the wound healing (35, 36). As these reasons we hypothesized that the basis of the epidural scarring begin early stage of wounding due to excessive iNOS and NO production. Therefore, in the current study, we used local aminoguanidine, a selective inhibitor of iNOS, to prevent epidural fibrosis.

Materials and Methods

The experiments were performed on 30 male Wistar rats ranging in weight from 225 to 250 g (mean weight 235 g) obtained from İnönü University, Animal Research Center. The rats were divided into three equal groups. One day before surgery, rats were let go hungry and pretreated with the antibiotic enrofloxacin (Baytril, 2.27 mg/kg sc; Bayer). Rats were anesthetized with intraperitoneal ketamine (60 mg/kg) and xylazine (6 mg/kg) and placed on a heated surgical table to maintaine body temperature of the animal at 37°C during surgical experiment. Under sterile conditions and a surgical microscope, the total laminectomy was performed on the vertebrae L3-L5 to fully expose the Dura mater in all rats.

The groups were as following: Group 1: Control (n=10); only a laminectomy was performed as described above. Group 2: Spongostan group (n=10); a Spongostan was soaked with saline solution and left on the Dura mater after laminectomy. Group 3: Spongostan plus aminoguanidine group (n=10); the aminoguanidine (50 mg/kg B.W.) soaked to spongostan andleft on the Dura mater after laminectomy. The wounds was closed with external sutures, and a topical antibiotic spray (Furazolidone aerosol powder) was applied to the external surface of the wound. The rats were allowed to recover on the heated table and closely observed for any signs of distress until awakening. The rats were observed about complications, wound infections, or any adverse effects observed relevant to aminoguanidine until they were sacrificed.

On the 14th day of laminectomy, all rats were sacrificed and vertebrae L3-L5 were removed as en bloc in a manner that included the paraspinal muscles. The specimens were fixed in 4% paraformaldehyde for 1 week, and and then were decalcified for 5 days in EDTA/Hydrochloric acid solution. The laminectomy site was identified and 2 mm thick sections were obtained. Sections were embedded in paraffin and serial sections (5 μm) were cut with microtome and stained with Hematoxylin Eosin (HE) for histopathological examination.

Histopathological study was performed on the transverse sections of laminectomized (L3-L5) areas for fibrosis by a pathologist.

Results

There was no complications, no wound infections, or any adverse effects observed relevant to aminoguanidine in rats. Overall histopathological results are shown in Figures 1-3. In the control group, there was obvious epidural fibrosis and compression. Epidural fibrosis almost covered the whole laminectomy defect and adhered to the underlying Dura mater. Moderate epidural fibrosis and compression were revealed in control spongostan group. In contrary, there was not any fibrous proliferation and compression in the spongostan plus aminoguanidine group.

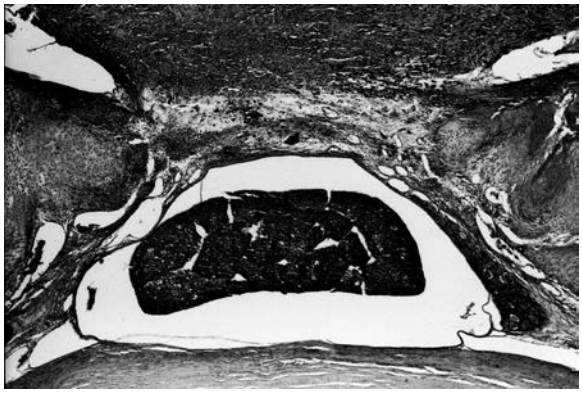

Figure 1. The control group (HE. x40).

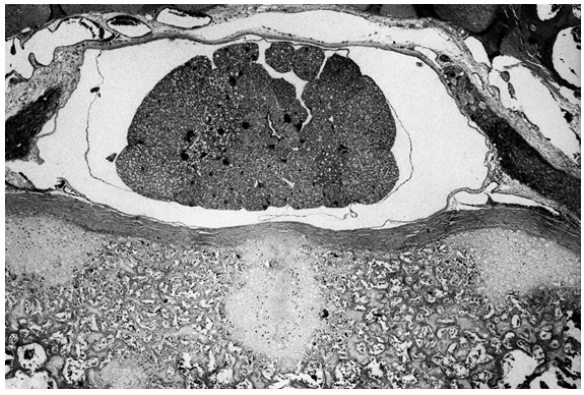

Figure. 2. The spongostan group (HE. x40).

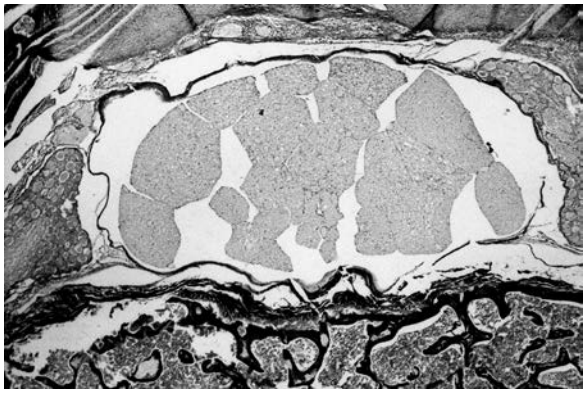

Figure 3. The spongostan plus aminoguanidine group (HE. x40).

Discussion

Epidural scarring and dural compression after laminectomy and/or discectomy causes compression to the dural sac and compression or traction to the nerve root which is probably the cause of recurrent low back and/or radicular pain (1, 2, 10, 11). On the other hand, the presence of scarring after laminectomy and/or discectomy makes the reoperation more difficult, and there is more risk of iatrogenic injury because of dural adhesion (3-5, 7-9, 11, 14, 16, 26, 28).

Although many synthetic and biological materials to prevent postlaminectomy scarring or fibrosis have been used experimentally and clinically, such as preclude spinal membrane, ADCON-L, Gelfoam polyglactin 910 mesh, Vicryl mesh, Avitene, Silastic, atoulogous fat graft or chemicals like dexamethasone. There is not universal consent about any of the methods (15-27). On the other hand, it has been shown that these materials, used to prevent postlaminectomy scarring, may also cause scarring themselves, because of foreign body reaction. Also, hypertrophic epidural scarring and dural compression were observed in some cases despite the presence of autologous fat grafts (26-28).

Many researchers reported that NO produced by iNOS has a very important role in early wound. Being a signal transducer, NO which is produced from l-arginine by iNOS, has a effect on wound healing as well. iNOS is frequently expressed in response to acute inflammatory stimuli after wounding. Expression or physiologic activity has been demonstrated in macrophages, lymphocytes, neutrophils, fibroblasts (29-34). Although the macrophage is the primary cell type implicated in iNOS activity and NO production during inflammation. Fibroblasts have also been shown to produce iNOS in the healing wound at a time that is coincidental with active collagen synthesis (31, 37). Significant expression of iNOS in the wound in the initial 24 to 72 hours after wounding is important for regulation of collagen accumulation and acquisition of mechanical strength. In contrary, there is a restricted expression of iNOS in the late wounds. This is also important for wound remodeling. Thus, it is thought that the basis of epidural scarring and Dural adhesion results from extensive iNOS expression, occurred in the early wound (38-42). In a similar study, it was shown that the inhibition of wound nitric oxide synthase by iNOS inhibitors was paralleled by lowered accumulation of collagen in wounds (43).

Aminoguanidine is a iNOS inhibitors (44). In our study, we used aminoguanidine as topically to prevent development of epidural fibrosis. Aminoguanidine was not any adverse effects on rats because of used locally and absorbed in short time. It was shown that there is not any fibrous proliferation or compression in the epidural space, in the topical aminoguanidine administrated group. In contrary there was obvious extradural fibrosis and compression in the control group.

Our results suggested that aminoguanidine can use locally to prevent epidural scarring and compression without any adverse effect on host.

Kaynaklar

1. Ben Debba M, Augustus van Aphen H, Long DM. Association between peridural scar and activity-related pain after lumbar discectomy. Neurol Res 1999; 21 (Supll 1): 537-42.
2. Boden SC, Wiesel SW. The multiply operated low back patient. In: Rothman and Simeone, eds. The spine. Philadelphia: Saunders Co 1992: 1899-906.
3. Ebeling U, Kalbarcyk H, Reulen HJ. Microsurgical reoperation following lumbar disc surgery. Timing, surgical findings, and outcome in 92 patients. J Neurosurg 1989; 70(3): 397-404.
4. Fritsch EW, Heisel J, Rupp S. The failed back surgery syndrome: reasons, intraoperative findings, and long-term results: a report of 182 operative treatments. Spine 1996; 21(5): 626-33.
5. Gabriel EM, Friedman AH. The failed back surgery syndrome in Neurosurgery. In: Wilkins RH, Rengachary SS, eds. New York: McGraw-Hill Health Professions Divisions 1996: 3863-70.
6. Guyer RD, Patterson M, Ohnmeiss DD. Failed back surgery syndrome: diagnostic evaluation. J Am Acad Orthop Surg 2006 Sep; 14(9): 534-43.

7. Kim SS, Michelsen CB. Revision surgery for failed back surgery syndrome. Spine 1992; 17: 957-60.
8. Kim CH, Chung CK, Park CS, Choi B, Kim MJ, Park BJ. Reoperation rate after surgery for lumbar herniated intervertebral disc disease: nationwide cohort study. Spine 2013; 38(7): 581-90.
9. Law JD, Lehman RA, Kirsch WM. Reoperation after lumbar intervertebral disc surgery. J Neurosurg 1978; 48(2): 259-63.
10. Maroon JC, Abla A, Bost J. Association between peridural scar and persisten low back pain after lumbar discectomy. Neurol Res 1999; 21(Supll 19: 543-6.
11. North RB, Campbell JN, James CS, et al. Failed back surgery syndrome: 5-year follow in 102 patients undergoing repeated operation. Neurosurgery 1991; 28: 685-90.
12. Ozer AF1, Oktenoglu T, Sasani M, Bozkus H, Canbulat N, Karaarslan E, Sungurlu SF, Sarioglu AC. Preserving the ligamentum flavum in lumbar discectomy: a new technique that prevents scar tissue formation in the first 6 months postsurgery. Neurosurgery 2006; 59(1 Suppl 1): 126-33.
13. Ozgen S, Naderi S, Ozek MM, Pamir MD. Finding and outcome of revision lumbar disc surgery. J Spinal Disord 1999; 12(4): 287-9.
14. Wenger M, Mariani L, Kalbarczyk A, Groger U. Long-term outcome of 104 patients after lumbar sequestrectomy according to Williams. Neurosurgery 2001; 49(2): 329-34.
15. de Divitiis E, Cappabianca P. Preserving the ligamentum flavum in lumbar discectomy: a new technique that prevents scar tissue formation in the first 6 months postsurgery. Neurosurgery 2007; 61(6): 1340.
16. Brotchi J, Pirotte B, De Witte O, Levivier M. Prevention of epidural fibrosis in a prospective series of 100 primary lumbo-sacral discectomy patients: follow-up and assessment at re-operation. Neurol Res 1999; 21(Suppl 1): 47-50.
17. Geisler FH. Prevention of peridural fibrosis: current methodologies. Neurol Res 1999; 1(Suppl 1): 9-22.
18. Gill GG, Sakowich L, Thompson EC. Pedicled fat grafts for the prevention of scar formation after leminectomy. An experimental study in dogs. Spine 1979; 4: 176-86.
19. Hinton JL Jr, Warejcka DJ, Mei Y, et al. Inhibition of epidural scar formation after lumbar laminectomy in the rat. Spine 1995; 20(5): 564-70.
20. Jacobs RR, McClain O, Neff J. Control of postlaminectomy scar formation. An experimental and clinical study. Spine 1980; 5: 223-9.
21. Kuivila Te, Berry JL, Bell GR, Steffee AD. Heparinized materials for the control of the formation of the laminectomy membrane in experimental laminectomies in dogs. Clin Orthop 1988; 236: 166-74.
22. Mohsenipour I, Daniaux M, Aichner F, Twerdy K. Prevention of local scar formation after operative discectomy for lumbar disc herniation. Acta Neurochir (Wien) 1998; 140 (1): 9-14 .
23. Tao H, Wang Q, LU R. Prevention of epidural scar adhesion following lumbar spine operation. Chung Hua Wai Ko Tsa Chih 1996; 34(6): 344-7.
24. Xiong M, Song Y, Liu L. An experimental study of prevention of peridural adhesion following laminectomy. Chung Kuo Hsiu Fu Chung Chien Wai Ko Tsa Chih 1998; 12(5): 272-5.
25. Lee Ck, Alexander H. Prevention of postlaminectomy scar formation. Spine 1984; 9: 305-12.
26. Martin-Ferrer S. Failure of autologous fat grafts to prevent postoperative epidural fibrosis in surgery of the lumbar spine. Neurosurgery 1989; 24(5): 718-21.
27. Nussbaum CE, McDonald JV, Baggs RB. Use of Vicryl (polyglactin 910) mesh to limit epidural scar formation after laminectomy. Neurosurgery 1990; 26: 649-54.
28. Mayer PJ, Jacopsen FS. Cauda equina syndrome after surgical treatment of lumbar spine stenosis with application of free autogenous fat graft. J Surg Bone Joint Surg (Am) 1989;71:1090-3.
29. Abo El-Noor MM, Elgazzar FM, Alshenawy HA. Role of inducible nitric oxide synthase and interleukin-6 expression in estimation of skin burn age and vitality. J Forensic Leg Med. 2017; 52: 148-53.
30. Diegelmann RF, Evans MC. Wound healing: an overview of acute, fibrotic and delayed healing, Front. Biosci 2004; 9: 283-9.
31. Efron D, Kirk SJ, Regan MC, Wasserkrug HL, Barbul A. Nitric oxide generation from L-arginine is required for optimal peripheral blood lymphocyte DNA synthesis. Surgery 1991; 110: 327-34.
32. McCall TB, Boughton-Smith NK, Palmer RM, Whittle BJ, Moncada S. Synthesis of nitric oxide from L-arginine by neutrophils. Release and interaction with superoxide anion. Biochem J 1989; 261: 293-6.
33. Rizk M, Witte MB, Barbul A. Nitric oxide and wound healing, World J. Surg. 2004;28(3):301-6.
34. Shi H P, Efron D T, Most D, Tantry U, and Barbul A. "The role of iNOS in wound healing," Surgery 2001; 130(2): 225-9,
35. Schaffer MR, Tantry U, Thornton FJ, Barbul A. Inhibition of nitric oxide synthesis in wounds: pharmacology and effect on accumulation of collagen in wounds in mice. Eur J Surg 1999; 165(3): 262-7.
36. Stallmeyer B; Kämpfer H; Kolb N; Pfeilschifter J; Frank S. The Function of Nitric Oxide in Wound Repair: Inhibition of Inducible Nitric Oxide-Synthase Severely Impairs Wound Reepithelialization. J Invest Dermatol 1999; 113(6): 1090-8.
37. Wang R, Ghahary A, Shen YJ, Scott PG, Tredget EE. Human dermal fibroblasts produce nitric oxide and express both constituve and inducible nitric oxide synthase isoforms. J Invest Dermatol 1996; 106(3): 419-27.

38. Albina JE, Mills CD, Barbul A, et al. Arginine metabolism in wounds. Am J Physiol 1988; 254: 459-67.
39. Krieg T, Braun-Falco O. Fibrocytes and fibroblasts. Definition and significance in wound healing and fibrotic disease of the skin. Z Hautkr 1989; 64 (9): 775-6.
40. Reichner JS, Meszaros AJ, Louis CA, et al. Molecular and metabolic evidence for the restricted expression of inducible Nitric oxide synthase in healing wounds. Am J Pathol 1999; 154(4): 1097-104.
41. Schaffer MR, Tantry U, Gross SS, Wasserburg HL, Barbul A. Nitric oxide regulates wound healing. J Surg Res 1996; 63(1): 237-40.
42. Schaffer MR, Efron PA, Thorton FJ, et al. Nitric oxide, an autocrine regulator of wound fibroblast synthetic function. J Immunol 1997; 158(5): 2375-81.
43. Tracey WR, Tse J, Cartere G. Lipopolysaccharide-induced changes in plasma nitrite and nitrate concentrations in rats and mice: pharmacological evaluation of nitric oxide synthase inhibitors. J Pharmacol Exp Ther 1995; 272: 1011-5.
44. Babu RB, Griffth OW. Design of isoform-selective inhibitors of nitric oxide synthase. Curr Opin Chem Biol 1998; 2: 491-500.

Sorumlu Yazar

M. Arif Aladag
Inönü University, Department of Neurosurgery, Turgut Ozal Medical Center, 44069 MALATYA, TURKEY
E mail: marifaladag@hotmail.com