Orijinal Araştırma

The Effects of Nitric Oxide on Extensive Epidural Fibrosis After Laminectomy in Rats: A Preliminary Study

Ratlarda Laminektomi Sonrası Aşırı Epidural Fibrozis Gelişmesinde Nitrik Oksitin Etkileri: Bir Ön Çalışma

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Özet

Amaç: Laminektomi ve/veya diskektomi sonrası, tüm önleyici işlemlere rağmen, hastaların bir alt grubunda aşırı epidural fibrozis gelişmektedir. Bizim hipotezimiz, laminektomi sonrası aşırı fibrosis gelişen hastalarda yara iyileşmesinin geç safhasında nitrik oksit (NO) üretiminin arjinaz tarafından inhibisyonunda bir defektin olduğu şeklindedir. Amacımız, bir hayvan laminektomi modelinde aşırı fibrozis gelişmesi üzerinde NO ve arjinaz'ın etkisini araştırmaktır.

Gereç ve Yöntem: Bu çalışmada, 50 erkek wistar rat beş gruba ayrıldı; tümüne lomber laminektomi yapıldı. Laminektomi sonrası 3. ve 12. gün arasında, 1. gruba (kontrol) serum fizyolojik, 2. gruba; indüklenebilir nitrik oksit sentazı (iNOS) uyarmak üzere lipopolisakkarit (LPS), 3. gruba LPS ve L-arginin (NOS substratı), 4. gruba gliseril trinitrat (NO donörü) ve 5. gruba L-valine (arjinaz inhibitörü) intraperitonal olarak verildi.

Sonuçlar: Hayvanların tümü 12. gün sakrifiye edildi ve hayvanların tümünde epidural fibrozis gross ve histolojik inceleme ile değerlendirildi. 2. grupta yara iyileşmesi ve fibrozis zayıf iken, 3'üncü grupta hafif derecede kompresyon yapan bir epidural fibrozis vardı. 4. ve 5'inci gruplarda ise ciddi derecede kompresyon yapan bir epidural fibrozis vardı.

Tartışma: iNOS inhibe edilerek NO üretiminin azalması ve L-arjinin'in büyük oranda arjinaz tarafından kullanılması, yara iyileşmesinin geç dönemindeki tamir ve yenilenme işlemini etkileyen en önemli faktörler olduğu kanaatindeyiz.

Anahtar Kelimeler: Rat, Laminektomi, Nitrik Oksit, Arjinaz, Epidural Fibrozis, Yara Iyileşmesi.

Abstract

Aim: In spite of all preventive procedures, extensive epidural fibrosis following laminectomy or/and dissectomy still remains a major problem. We hypothesized that the patients, who suffered from extensive fibrosis, have a defect in inhibition of nitric oxide (NO) production that make increase in fibroblastic activity and collagen accumulation in healing wound. Aim of this study is to explore the effect of NO and arginase on extensive fibrosis in an animal model of laminectomy.

Material and Method: 50 male wistar rats are divided in five groups; all of them were made laminectomy in lumbar spine. Between the 3rd and 12th days, we gave saline to the group1 (control group), lipopolysaccharide (LPS) to the group 2 to induce inducible nitric oxide synthase (iNOS), LPS plus L-arginine (NOS substrate) to the group 3, glyceryl trinitrate (NO donor) to the group 4 and L-valine (an arginase inhibitor) to the group 5 intraperitonally.

Results: All of these animals were sacrificed on the 12th day after operation, and the epidural fibrosis of every animal was evaluated by gross observation and histological examinations. While in the group 2 wound healing and fibrosis was poor, in the group 3, there was mild degree epidural fibrosis, but epidural fibrosis was not compressive. In group 4 and 5 there were severe degree compressive extensive epidural fibrosis.

Conclusion: It was concluded that the reduction of NO production by inhibiting iNOS and largely use of L-arginine by arginase are the most important factors that affect the process of repair and remodeling in the late period of wound healing, therefore, the absence or insufficiency in the NOS inhibition or extremism in NO production during late period of the wound healing leads to extensive epidural fibrosis.

Keywords: Rat, Laminectomy, Nitric Oxide, Arginase, Epidural Fibrosis, Wound Healing.

Introduction

Although, epidural scarring and dural adhesions following lumbar laminectomy was decreased by advances in surgical techniques and preventive methods, there is a subgroup patients experiencing extensive epidural fibrosis after laminectomy or discectomy from which unfavorable results were noted with most reoperations. Being a natural consequence of the

normal post-operative healing, epidural fibrosis why occasionally occurs in extensive amounts and involve in spinal canal has not been clearly explained (1-3). Studies suggested that in wound healing, while NO production from the expression of iNOS is critical to in early stage, the inhibition of NO production by increased arginase activity and by suppression of iNOS induction is important in the processes of repair and remodeling in the late stage of wound healing (4-7).

On the basis of these informations, we hypothesized that the possible cause of extensive epidural fibrosis could be the extensive production of NO due to absence or insufficiency in inhibition of NO production in late wound. Hence, we investigated the effect of NO wound healing and extensive epidural fibrosis a laminectomy model in rats.

Material And Method

Experiments were performed on 50 male Wistar rats ranging in weight from 225 to 250 g (mean weight of 235 g) obtained from Inönü University, animal research laboratory. Wistar rats laminectomized from L5 to L6 and were divided into five groups: group 1, injected saline (1 ml 0.9% NaCl) one in every day; group 2, injected LPS (4 mg/kg, Sigma; pseudomonas aeruginosa serotype 10) one in every other day for seven days; group 3, injected LPS (4 mg/kg) one in every other day plus L-arginine (200 mg/kg) in every day for seven days; group 4, injected gyceryl trinitrate (2.5 mg/kg) three times in a day for seven days; and group 5, injected L-Valine (300 mg/kg) in every day. All injections were made intraperitonally, from 3rd day to 10 ^{the} after laminectomy.

Laminectomy procedure: An animal model of laminectomy in rats was used to study scar tissue formation around the spinal cord. Laminectomy experiment in rat was performed on the vertebrae L5-L6 to expose the spinal cord. 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 maintained body temperature of the animal at 37°C during surgical experiment. Under sterile conditions and a surgical microscope, we made a midline incision and exposed the midthoracic cord by laminectomy at the level of vertebrae L5-L6.

The exposure 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.

12th day of laminectomy, all of the rats were killed and vertebrae L5-L6 were removed completely. Then the tissues were fixed in paraformaldehyde %4 and sectioned for histopathological examination. Histopathological study was performed on the transverse sections of laminectomized (L5-L6) areas and graded by a pathologist. Each specimen was scored for extent, density, and epidural involvement by fibrosis.

Results

Overall histopathological results are shown in Figures 1-5. In the control group (Fig.1) there was not any compression on thecal sac.

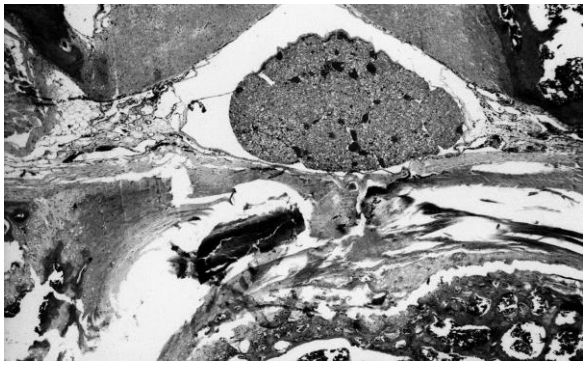

Figure 1. In saline injected group (Group 1.), histopathologic observation of epidural fibrosis harvested on the 12th day after laminectomy (H&E x10). Wound healing and remodeling are normal.

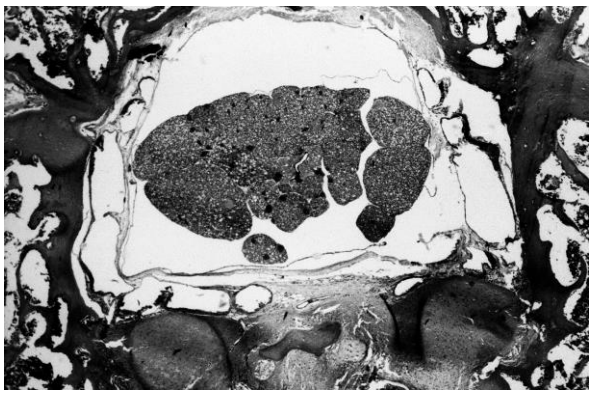

Figure 2. In LPS injected group (Group 2.), histopathologic observation of epidural fibrosis harvested on the 12th day after laminectomy (H&E x10). Wound healing and remodeling are poor.

While in the only LPS given group wound healing and fibrosis was poor (Fig. 2), there was mild degree fibrous proliferation in the epidural cavity and was not any compressive effect on thecal sac in the group given LPS and plus l-arginine (Fig. 3).

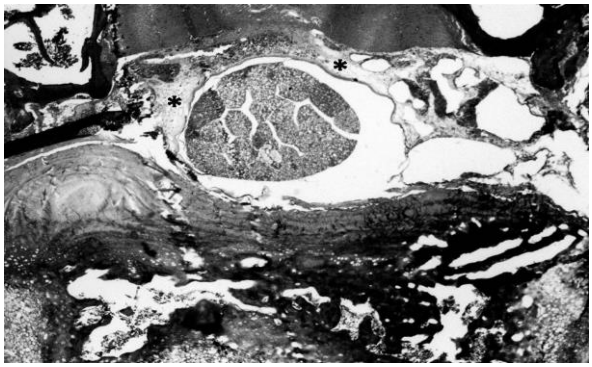

Figure 3. In LPS plus L-arginine injected group (Group 3.), histopathologic observation of epidural fibrosis harvested on the 12th day after laminectomy (H&E x10). There is mild degree epidural fibrosis that has not compressive effect on thecal sac. *Moderate epidural fibrosis.

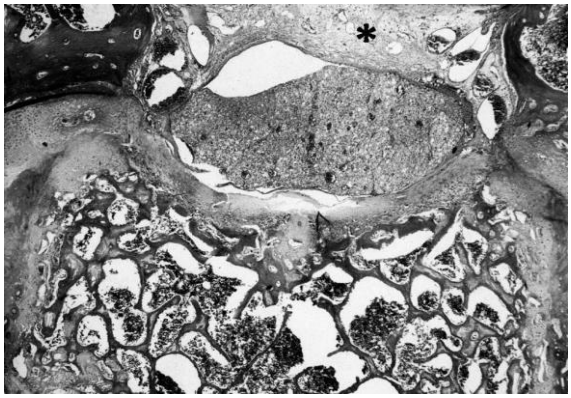

Figure 4. In Glyceryl trinitrate injected group (Group 4.), histopathologic observation of epidural fibrosis harvested on the 12th day after laminectomy (H&E x10). Epidural fibrosis is greater than LPS and plus l-arginine injected group and has severe degree compressive effect on thecal sac.
*Excessive epidural fibrosis.

Figure 5. In L-Valine injected group (Group 5.), histopathologic observation of epidural fibrosis harvested on the 12th day after laminectomy (H&E x10). Epidural fibrosis and compressive effect is greater than Glyceryl trinitrate injected group. *Excessive epidural fibrosis.

In contrast, there was severe degree compressive extensive epidural fibrosis in glyceryl trinitrate (a NO donor) and L-valine (an arginase inhibitor) given groups (Figs. 4 and 5). Epidural fibrosis was greater in L-valine given group than in the NO donor given group.

Discussion

Epidural fibrosis is a natural consequence of the normal post-operative healing. It occasionally can cause an adhesion of dura or root or a mass with extensive epidural fibrosis with the invasion of the scar tissue in the spinal canal which it displaces nerve root, and conceivably be the source of pain and cause of failed back surgery syndrome (1-3, 8).

Although, many attempt to prevent or reduce epidural scar formation, using many material in the prevention of postoperative epidural adhesion or resorbable barrier to epidural fibrosis and to improve surgical techniques, reduce the rate of epidural fibrosis, there is a group of patient who was suffered from extensive epidural fibrosis after laminectomy and/or discectomy in spite of all precautions. In this regard, very unfavorable results were noted with most reoperations of these patients (2, 3, 8-15).

Being a signal transducer, NO which is produced from L-arginine by inducible nitric oxide synthase (iNOS), has an effect on wound healing as well. The data of many studies collectively suggest that nitric oxide synthesis is critical to wound collagen accumulation and acquisition of mechanical strength. Inducible NOS is frequently produced in response to acute inflammatory stimuli after wounding. Expression or physiologic activity of inducible NOS has been demonstrated in macrophages, lymphocytes, neutrophils, fibroblasts (4, 16-24). Although the macrophage is the primary cell type implicated in inducible NOS activity and NO production during inflammation, NO production by dermal fibroblasts is especially important during the inflammatory stages of wound healing and possibly also in the later stages of proliferation and tissue remodeling with active collagen synthesis (4, 25). It was shown that NO production by dermal fibroblasts is important during the inflammatory stages of wound healing and possibly also in the later stages of proliferation and tissue remodeling after skin injury in humans.

Wound fibroblasts (WS) are phenotypically altered during the healing process to synthesize NO, which, in turn, regulates their collagen synthesis and contractile activities (20, 25, 26). Arginase share a common substrate L-arginine with NOS and the increase of arginase activity results in the decrease of NO production. Therefore arginase regulate NO production (27-29). LPS induced both iNOS and arginase

activities (27, 28). The data obtained many studies also shown more detail knowledge about the role of NO in wound healing. These studies demonstrated that iNOS activity was highest in wounds first 6 to 24 hours after injury, then iNOS activity began to decrease while arginase activity began to increase and this reduction continued up to 48 to 72 hours. These findings indicate us that reverse the balance between NO production and arginase activity to favor of arginase from favor of NO in late phase of wound. This situation is important in remodeling of wound (4-7, 16, 21-26).

In our study, in only LPS given group wound healing and remodeling was poor, in LPS plus L-arginine given group there was moderate epidural fibrosis, but there was no any compressive effect of on thecal sac. In contrast to, in group 4 that administered gliseryl trinitrate (a NO donor) and in group 5 that L-valine (arginase inhibitor) given group, there was severe degree compressive extensive fibrosis. But the degree of epidural fibrosis in L-valine given group was greater than in the NO donor given group. Our results consistent with the studies that indicate the importance of NO in wound healing (4, 14-26).

In our study, rats given LPS and plus l-arginine exhibit mild epidural fibrosis involving the spinal canal because of LPS inducted both iNOS and arginase activities simultaneously. Arginase and iNOS compete for the use of L-arginine, so NO synthesis is reduced. In contrast, rats given glyceryl trinitrate (NO donor) and L-valine (an arginase inhibitor) showed severe degree compressive extensive epidural fibrosis development due to excessive NO production in both group. Contrarily to these groups, only LPS given rats exhibit poor wound healing and epidural fibrosis because an increased consumption of L-arginine by arginase results in down regulation of NO production by decreasing intracellular L-arginine concentrations in early and later wound. Because, it was showed that besides NO induction, LPS also caused an important increase in arginase activity in macrophages (27-29).

Although, our study is limited only histopathological data, our results suggested that the importance of variable balance between NO and arginase and, indicated that changing this balance in favor of NO instead of arginase in late wound may cause a development of extensive epidural fibrosis.

As a result, we concluded that overproduction of nitric oxide, probably due to insufficient inhibition of iNOS in late wound, plays an important role in pathophysiology of extensive epidural fibrosis after laminectomy. However, further studies are necessary to clarify the exact role of NO on developing extensive epidural fibrosis in late wound after laminectomy.

References

1. 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.
2. Cekinmez M, Sen O, Atalay B, Erdogan B, Baybek M, Caner H, et al: Effects of methyl prednisolone acetate, fibrin glue and combination of methyl prednisolone acetate and fibrin glue in prevention of epidural fibrosis in a rat model. Neurol Res 2010; 32: 700-5.
3. 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.
4. Schaffer MR, Tantry U, Gross SS, Wasserkrug HL, Barbul A: Nitric oxide regulates wound healing. J Surg Res 1996, 63: 237-40
5. Alexander JW, Supp DM. Role of Arginine and Omega-3 Fatty Acids in Wound Healing and Infection. Adv Wound Care (New Rochelle) 2014; 3(11): 682-90.
6. Stechmiller JK, Childress B, Cowan L. Arginine supplementation and wound healing. Nutr Clin Pract 2005; 20(1): 52-61.
7. Lee RH, Efron D, Tantry U, Barbul A. Nitric oxide in the healing wound: A time-course study. J Surg Res 2001; 101: 104–8.
8. Tao H, Wang Q, LU R. Prevention of epidural scarr adhesion following lumb spine operation. Chung Hua Wai Ko Tsa Chih 1996; 34(6): 344-7.
9. Burton CV, Kirkaldy-Willis WH, Yong-Hing K, et al. Causes of failure of surgery on the lumbar spine. Clin Orthop 1981; 157: 191-9.
10. Finnegan WJ, Fenlin JM, Marvel JP, et al. Results of surgical intervention in the symtomatic multiply-operated back patient: analysis of sixty-seven cases followed for three to seven years. J Bone Joint Surg (Am) 1979; 61-A: 1077-82.
11. Gabriel EM, Friedman AH. The failed back surgery syndrome in Neurosurgery. In: Wilkins RH, Rengachary SS, eds. New York, St. Louis, San Fransisco: McGraw-Hill Co, Health Professions Division 1996; 2nd edition, Vol. IIIB: 3863-70.
12. Kim SS, Michelsen CB. Revision surgery for failed back surgery syndrome. Spine 1992; 17: 957-60.
13. Nussbaum CE, McDonald JV, Baggs RB. Use of Vicryl (polyglactin 910) mesh to limit epidural

scar formation after laminectomy. Neurosurgery 1990; 26: 649-54.
14. Robertson JT, Maier K, Anderson RW, Mule JL, Palatinsky EA. Prevention of epidural fibrosis with ADCON-L in presence of a duratomy during lumbar disc surgery: experiences with a preclinical model. Neuro Res 1999; 21 (Suppl 1): 561-6.
15. Vural E, Yilmaz M, Ilbay K, Ilbay G. Prevention of Epidural Fibrosis in Rats by Local Administration of Mitomycin C or Daunorubicin. Turk Neurosurg 2016; 26(2): 291-6.
16. Thorton FJ, Schaffer MR, Witte MB, et al. Enhanced collagen accumulation following direct transfection of the inducible nitric oxide synthase gene in cutaneous wounds. Biochem Biophys Res Commun 1998; 246 (3): 654-9.
17. 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: 27-334.
18. 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.
19. Schaffer MR, Tantry U, Van Wesep RA, Barbul A. Nitric oxide metabolism in wounds. J Surg Res 1997; 71(1): 25-31.
20. 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.
21. 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.
22. Stuehr DJ, Marletta MA. Induction of nitrite/nitrate synthesis in murine macrophages by BCG infection, lymphokines, or interferon-[gamma]. J Immunol 1987; 140: 518-25.
23. 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.
24. Werner-Felmayer G, Werner ER, Fuchs D, Hausen A, Reibnegger G, Wachter H. Tetrahydrobiopterin-dependent formation of nitrite and nitrate in murine fibroblasts. J Exp Med 1990; 172: 1599-607.
25. Witte MB, Schäffer MR, Barbul A. Phenotypic induction of nitric oxide is critical for synthetic function in wound fibroblasts. Surg Forum 1996; 47: 703-5
26. 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): 775-81.
27. Durante W, Johnson FK, Johnson RA. Arginase: a critical regulator of nitric oxide synthesis and vascular function. Clin Exp Pharmacol Physiol 2007; 34(9): 906-11.
28. Klasen S, Hammermann R, Fuhrmann M, Lindemann D, Beck KF, Pfeilschifter J, Racke K. Glucocorticoids inhibit lipopolysaccharide-induced up-regulation of arginase in rat alveolar macrophages. Br J Pharmacol 2001; 132(6): 1349-57.
29. Imagama T, Ogino K, Takemoto K, Kato Y, Kataoka H, Suzuki H, Ran Z, Setiawan H, Fujikura Y, Taguchi T. Regulation of nitric oxide generation by up-regulated arginase I in rat spinal cord injury. J Clin Biochem Nutr 2012; 51(1): 68-75.

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