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Original Article

Preventive effect of dexamethasone gelatin sponge on the lumbosacral epidural adhesion

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Abstract: Objective: This study aims to explore the preventive effect of dexamethasone gelatin sponge on the lumbosacral epidural adhesion in the laminectomy. Methods: A total of 36 Wista rats were divided into A, B, C and D groups randomly. Dexamethasone was not used in group A, Dexamethasone was used in group B, Dexamethasone was not used in group C but covered with gelatin sponge, dexamethasone gelatin sponge was used in group D. 3 rats in each group were sacrificed at 4, 8 and 12 weeks after operation respectively and the wound was opened to observe the dural scar formation and the dura adhesion. Immunohistochemical technique was used for histology observation. The expressions of VEGF and VEGFR2 in the epidural scar and surrounding tissues were detected with western blotting and immunohistochemical methods. Results: According to the Rydell score standard, there were different degree of adhesion formation in A, B and C groups while there was no obvious adhesion formation in D group. It was confirmed that the expressions of VEGF and VEGFR2 in group D were lower than that of the other groups. Conclusions: Dexamethasone gelatin sponge could significantly reduce the occurrence of epidural scar tissue hyperplasia and adhesion after laminectomy in rats, and its mechanism may be related to the decreased expression of VEGF and VEGFR2.

Keywords: Dexamethasone, gelatin sponge, epidural adhesion, VEGF, VEGFR2

Introduction

Dural fibrosis and epidural adhesion after laminectomy are developed from adjacent dense scar tissue, which is a natural wound healing process [1-4]. The proliferative cells are the fibroblasts near the spinal muscle tissues. The pathological scar will develop to hypertrophy capsule and form membranous tissue after laminectomy. This phenomenon was found by LaRocca and Macnob in 1974 [5]. The epidural hyperplasia can extend to the spinal canal and adhere to the dura and nerve root, which lead to the periodic recurrent symptoms including nerve root pain [6-10]. At the same time, dural fibrosis made the risk of nerve root injury, dural laceration and latrogenic injury greatly improved, which challenging doctors for exploring the operation technology [13-15].

The prevention of scar formation has become a hotspot in spinal operation. A series of biological, pharmaceutical and synthetic materials are used to study the prevention of scar formation after laminectomy. Silicone rubber, polyester materials, fat transplantation, cholesterol, mitomycin C, recombinant tissue plasminogen activator and anti-inflammatory drugs were used to study, but the results were not consistent [16-22].

The use of physical cover to protect tissue surface is conducive to wound healing, but the possibility of the clinical practice remains to be further studied. The purpose of this study was to evaluate the effect of dexamethasone gelatin sponge on the vertebral plate reconstruction in rat laminectomy model and observe whether it can reduce the epidural adhesion effectively.

Materials and methods

Experimental animals

A total of 36 Wista rats (weight 260-280 g) were divided into 4 groups with the method of ran-

Table 1. Rydell score of general observation

Group	Rydell score
A	2.90 ± 0.11
B	2.93 ± 0.22
C	1.24 ± 0.12*
D	1.25 ± 0.18*

*Compared with control group, $P < 0.01$.

dom number: Dexamethasone was not used and no medium covered (group A), dexamethasone was used (group B), dexamethasone was not used but covered with gelatin sponge (group C), dexamethasone gelatin sponge was used (group D). Each group has 9 rats. Laminectomy was undergone in each animal. The rats were anesthesia with intraperitoneal injection of 35 mg/kg ketamine and 5 mg/kg xylazine. Intraperitoneal injection of 75 mg/kg cefazolin was performed before operation to prevent infection. The formation of 5 × 2 mm laminectomy damage in L2 and L4 was carried out after detachment of paravertebral muscles and resection spinous process, the spinal membrane was cleaned and fully exposed, hemostasis by light pressing with absorbent cotton. The exposed dura and nerve root were stitched directly layer by layer in group A. In the nerve root site 1 ml of dexamethasone was injected before the suture of wound in group B. Laminectomy damage site was covered with gelatin sponge before the suture of wound in group C. Laminectomy damage site was covered with gelatin sponge infiltrating dexamethasone before the suture of wound in group D. Dexamethasone (Drug approval No H1202515) was purchased from Tianjin Jinyao amino acid Co., Ltd (Tianjin, China), gelatin sponge (Drug approval No H320224096) was purchased from Nanjing Jinling pharmaceutical limited Co., Ltd (Nanjing, China).

Housing and procedures involving experimental animals were in accordance with the Guide for the Care and Use of Laboratory Animals (eighth edition, published by the National Academies Press). All animal experiments were approved by the Animal Care and Studies committee of Southern Medial University.

Adhesion score

All animals were fed in different cages until the time of need. The physical status and neurological function of animals were observed in the recovery period. 3 rats in each group were sacrificed at 4, 8 and 12 weeks after operation respectively, the test spines including sacrospinal muscle were removed to observe the dural scar formation and the dural adhesion at nerve root with the naked eyes. The result was expressed as the scores according to the Rydell score standard.

H&E staining

The tissue samples were fixed in formalin for 48 h, and then they were decalcified with 10% nitrate for 2 weeks and dehydration. They were embedded in paraffin and the paraffin blocks of specimen were cut into continuous sections with 5 μm respectively. The sections were dewaxed with xylene and washed in various levels of ethanol and finally with water. They were stained with hematoxylin after that and then differentiated, washed and stained with eosin, then dehydrated, hyalinized and finally mounted on slides and observed under microscope. The result of immunohistochemical staining is an outcome of comprehensive evaluation which was scored by the pathologist and authors separately to minimize the bias. The result was expressed as the staining index scores according to the modified Nussbaum tissue score standard.

Detection of the expression of VEGF and VEGFR2 by western blotting and immunohistochemical methods

Western blotting: Briefly, tissues were lysed in lysis buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1 mM EDTA and protease inhibitor for proteins extracts. Total proteins were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred to a polyvinylidene difluoride membrane. Membrane was incubated with anti VEGF and VEGFR2 antibodies (1:1000) respectively and incubated at 4°C overnight. The secondary antibodies were added at the dilution of 1:1000 and bands were stained with DAB.

Immunohistochemistry: Following deparaffinization and dehydration, the sections underwent 0.5% potassium citrate microwave antigen retrieval at 100°C for 15 min. The sections were washed with TBS and then blocked with

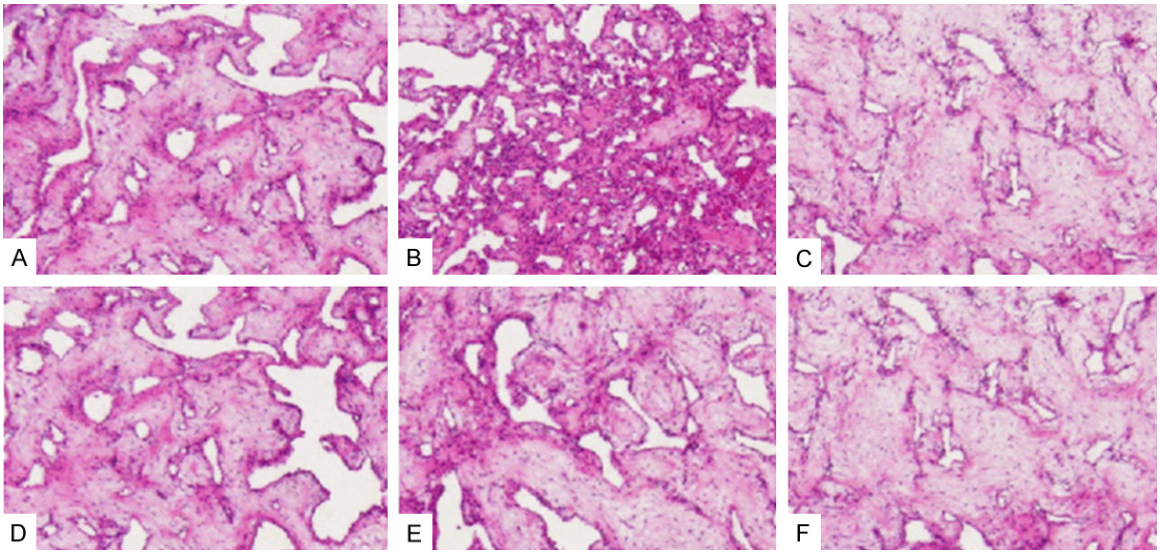

Figure 1. Results of H&E staining in group A and B. A: 4 weeks after operation in group A; B: 8 weeks after operation in group A; C: 12 weeks after operation in group A; D: 4 weeks after operation in group B; E: 8 weeks after operation in group B; F: 12 weeks after operation in group B.

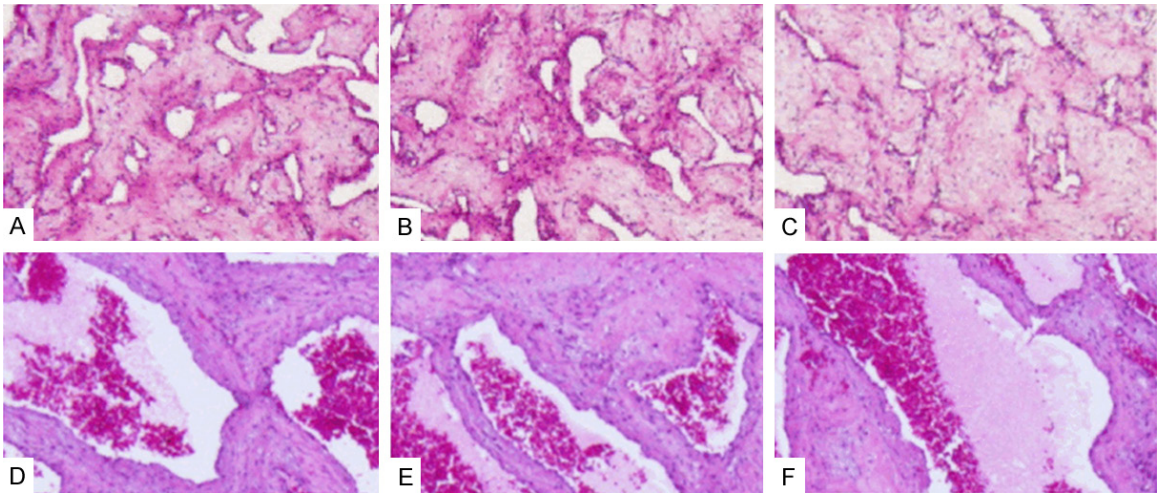

Figure 2. Results of H&E staining in group C and D. A: 4 weeks after operation in group C; B: 8 weeks after operation in group C; C: 12 weeks after operation in group C; D: 4 weeks after operation in group D; E: 8 weeks after operation in group D; F: 12 weeks after operation in group D.

5-10% goat serum and incubated at 37°C for 20 min.

The primary antibodies (1:200 VEGF and VEGFR2 antibodies) were subsequently added and incubated at 4°C overnight. The samples were then washed with PBS and incubated with secondary antibody (biotin-labeled goat anti-rabbit immunoglobulin G) at 37°C for 30 min. They were re-washed with PBS. Following treatment with 3,3'-diaminobenzidine solution, the sections were flushed, counterstained with hematoxylin, washed with water, dehydrated, cleared, mounted on slides and observed under the microscope.

Statistical analysis

Values are presented with mean ± SD. All data analysis was performed with SPSS 17.0 software. The data were analyzed with rank test. $P < 0.05$ was considered to be statistical significance.

Table 2. Nussbaum score of HE staining

Group	Nussbaum score
A	2.81 ± 0.15
B	2.73 ± 0.16
C	1.62 ± 0.13*
D	1.54 ± 0.11*

*Compared with control group, $P < 0.01$.

Results

General observation and Rydell score

In group A, it was seen with the naked eyes that the epidural covering in laminectomy site implanted into the surrounding tissue and spinal canal, the scar tissue in the wound surface proliferated obviously and adhered densely, it was difficult for blunt dissection and dura could not keep complete separation; the implantation in group B was similar to that of group A. There was mild adhesion between scar tissue and dura in laminectomy site in group C and could be separated with operation instrument. There was proliferation of scar tissue in the wound surface and it was capable of blunt separation of intact dura with operation apparatus.

Rydell score showed that there was no significant difference between group A and B ($P > 0.05$) while there was significant difference between group A and C, group A and D respectively ($P < 0.01$, **Table 1**).

H&E staining

In group A at 4 weeks after operation, there was serious inflammatory infiltration, the epidural clearance was reduced but no formation of oppression; there was fibroblast hyperplasia, fibrosis and dense collagen and adhesions at 8 weeks after operation; there was severe adhesion at 12 weeks after operation (**Figure 1A-C**). In group B at 4 weeks after operation, there was mild adhesion and a small amount of fibroblasts and inflammatory cells hyperplasia; there was increased fibroblast at 8 weeks after operation; there was fibrosis and severe adhesion of scar tissue to the dura at 12 weeks after operation (**Figure 1D-F**). In group C at 4 weeks after operation, there was inflammatory cell infiltration, capillary hyperplasia, part of gelatin sponge to be absorbed and scattered adhesion; the inflammatory cells decreased and fibrosis occurred at 8 weeks after operation; the dura thickened and adhered at 12 weeks after operation (**Figure 2A-C**). In group D at 4 weeks after operation, there was a small amount of inflammatory cells and obvious gap between the dural and gelatin sponge; there was osteoblast proliferation and no visible inflammatory cells at 8 weeks after operation; there was no scar formation and adhesion at 12 weeks after operation (**Figure 1D-F**).

The results of Modified Nussbaum score was shown in **Table 2**. It suggested that there was no significant difference between group A and B ($P > 0.05$) while there was significant difference between group A and C, group A and D respectively ($P < 0.01$).

Western blotting results of the expressions of VEGF and VEGFR2

The western blotting results of the expressions of VEGF and VEGFR2 were shown in **Figure 3**. It was found that VEGF and VEGFR2 expressed in all the samples of each group. However, the expression levels of VEGF and VEGFR2 in group C and D were significant lower than that of group A.

Immunohistochemical results of the expressions of VEGF and VEGFR2

The immunohistochemical results of the expressions of VEGF and VEGFR2 were shown in **Figure 4**. It showed that the expression levels of VEGF and VEGFR2 in group D were significant lower than that of group A. It was consistent with that of western blotting results.

Discussion

The formation of dense and thick epidural scar tissue is an important reason for poor prognosis of laminectomy, which could accompany with low back pain and some other side effects. Epidural fibrosis and adhesion is often due to dural injury caused by operation. Epidural adhesion can inhibit nervous activities and increase nervous tension in movement and cause nerve injury. Although the epidural scar tissue adhesion can be removed and the adhered nerve root can also be released, but this need second operation [23-25]. Patients may face more severe pathological symptoms after operation.

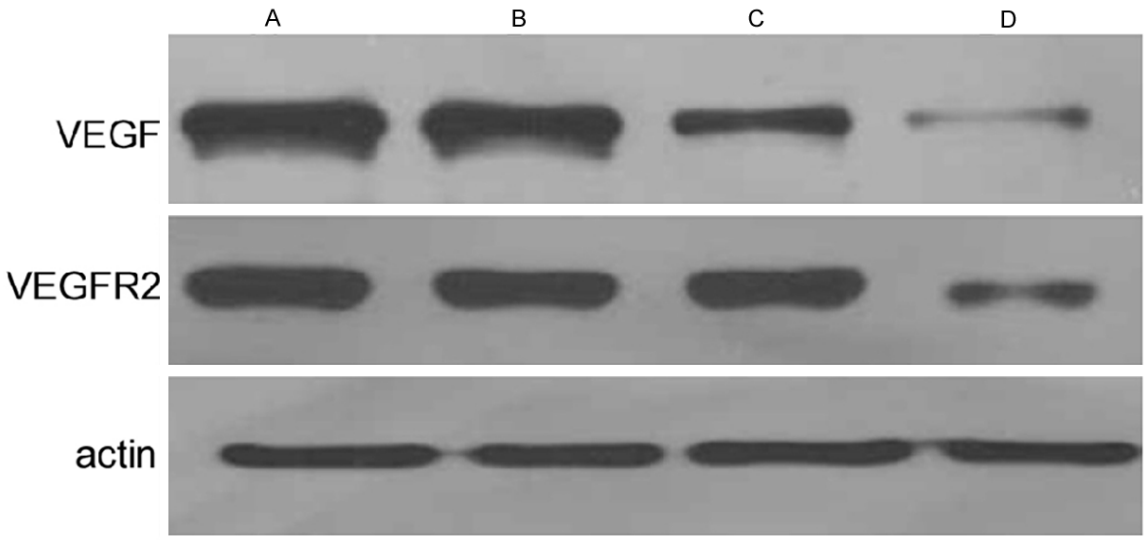

Figure 3. Western blotting results of the expressions of VEGF and VEGFR2 in different groups. A: Dexamethasone was not used and no medium covered group; B: Dexamethasone was used group; C: Dexamethasone was not used but covered with gelatin sponge group; D: Dexamethasone gelatin sponge was used group.

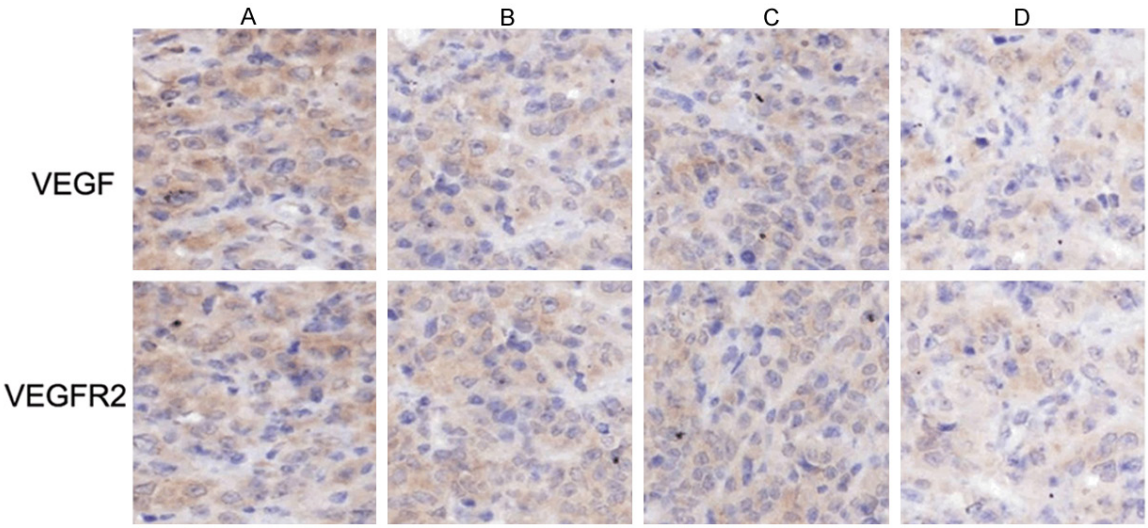

Figure 4. Immunohistochemical results of the expressions of VEGF and VEGFR2 in different groups. A: Dexamethasone was not used and no medium covered group; B: Dexamethasone was used group; C: Dexamethasone was not used but covered with gelatin sponge group; D: Dexamethasone gelatin sponge was used group.

Muscles covered spine and hematoma after operation may cause the dural adhesion and may be the main component of scar tissue. Therefore, to find a method or a medium to prevent the scar formation and the dura adhesion effectively and not affect the normal wound healing process in laminectomy has become a complicated task recently. Researchers tried to reduce scar formation through various operation techniques such as minimally invasive discectomy, application of local anti-inflammatory drugs, but the effects of these methods were not uniform [26-30].

In this study, we found that dexamethasone gelatin sponge could obviously inhibit the inflammatory reaction of operation site, and inhibit scar formation and adhesion. The soft tissue healing well in the implanted position and the inflammatory reaction was obviously mild compared with the control group. The results indicated that dexamethasone gelatin

sponge is a composite of relative security and accepted for experimental animal.

VEGF affected on vascular endothelial, which can increase vascular permeability and promote angiogenesis. Generally, the scar tissue has a rich vascular network. The increased expression of VEGF in scar tissue indicated that angiogenesis appeared in scar tissue. At the same time, VEGF may transmit information through VEGFR2 to regulate a series of physiological and pathological process [31, 32]. In this study, the expression of VEGF and VEGFR2 decreased after application of dexamethasone gelatin sponge, which may be one of the mechanisms of dexamethasone gelatin sponge preventing the scar formation and dural adhesion

In summary, this study demonstrated that the gelatin sponge could reduce the formation of epidural scar adhesion after laminectomy, the composite materials of dexamethasone gelatin sponge can promote this effect. The composite materials were relatively safe and experimental animals showed no adverse reaction. The soft tissue healed well in the covering sites and chronic inflammatory process was inhibited.

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Disclosure of conflict of interest

None.

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