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Reduction of epidural scar adhesion by topical application of simvastatin after laminectomy in rats

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Abstract. – OBJECTIVE: Epidural scar adhesion is one of the major causes of the failed back surgery syndrome after laminectomy. Recent studies have shown that simvastatin has potent anti-fibrotic and anti-inflammatory properties. This study evaluates the effect of the topical application of simvastatin on reducing epidural scar adhesion after laminectomy in rats.

MATERIALS AND METHODS: Thirty-six male Sprague-Dawley rats were randomly divided into three groups: control, chitosan and simvastatin group. After laminectomy was performed at L1 level, simvastatin and chitosan were applied to the laminectomy sites. The control group received no additional treatment. Four weeks later, the rats were killed and the epidural adhesion was evaluated by macroscopic assessment, hydroxyproline content analysis and histological analysis. The number of fibroblasts and the optical density of the collagen were also determined.

RESULTS: The results showed that simvastatin could reduce epidural scar adhesion in rats. Little epidural adhesions were seen in the laminectomy sites treated with simvastatin. The hydroxyproline content, the number of fibroblasts and the optical density of the collagen in the simvastatin group were significantly less than those of the chitosan and control group. However, dense epidural adhesion was found in control group.

CONCLUSIONS: Topical application of simvastatin could reduce epidural scar adhesion after laminectomy in rats. Further research is necessary to determine the optimal dosage and the safety of simvastatin.

Key Words:
Simvastatin, Epidural adhesion, Scar, Laminectomy, Rat.

Introduction

Epidural scar adhesion is the serious postoperative complication after lumbar surgery, which can compress the dura mater and restrict nerve root mobility[1,2]. Epidural scar adhesion makes the patients suffer from chronic nerve radicular or low back pain and results in failed back surgery syndrome[3,4]. Because complications such as dural tears, nerve root injury and bleeding may occur in revision spine surgery, the outcome of re-operation for epidural adhesion is unsatisfactory[5].

Many strategies have been used to prevent scar formation and reduce epidural adhesion. For example, microsurgical technique and good hemostasis have been applied to reduce scar formation after laminectomy. Many biological or non-biological materials have been implanted into laminectomy defects as a barrier to prevent epidural scar adhesion[6-8]. Many agents such as mitomycin C and steroid hormone are also used to reduce epidural scar adhesion[9,10]. However, the results are controversial and complete prevention of the epidural scar adhesion has not yet been achieved. Many treatments even affect the wound healing process.

Simvastatin, one of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, has been used as a lipid-lowering agent[11,12]. Many evidences have shown its beneficial effects on cardiovascular diseases in clinical trials[13,14]. Recent studies show the pleiotropic effects of simvastatin, such as anti-inflammatory and anti-fibrotic properties[15-17].

In this study we investigated the effect of simvastatin on reducing epidural scar adhesion by focusing on the pathological changes in the rat model, which may be useful in future human trials.

Materials and Methods

Thirty-six healthy male Sprague-Dawley rats, weighing 250 to 300 g, were purchased from the

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laboratory animal center of Yangzhou University. All animals were treated in compliance with the principles of Laboratory Animal Care, and the experimental protocol was approved by the Animal Care and Research Committee of the First Affiliated Hospital of Nanjing Medical University, China. The rats were divided into three groups: control (n=12); chitosan (n=12) and simvastatin (n=12). Before the experiment, the rats were housed for a week to adapt to the condition of the laboratory.

Simvastatin was purchased from Abcam, Cambridge, UK. Chitosan was obtained from Shanghai Qisheng Biologicals Co. Ltd., China. β-dimethylaminobenzaldehyde was purchased from Sigma (Saint Louis, MO, USA).

Rat laminectomy model were performed according to the procedure as described previously[18]. After anesthesia by intraperitoneal injection of pentobarbital (50 mg/kg body weight), the hairs around the location of L1 were shaved. The L1 vertebral plate was exposed by a midline skin incision and separation of the paraspinal muscles. The dura mater was exposed after removing the spinous process and vertebral plate of L1 by rongeur. A total laminectomy of L1 was performed.

After satisfactory hemostasis, simvastatin (1 mg/ml) was applied with chitosan to the laminectomy sites in simvastatin group. Only chitosan was applied to the laminectomy sites in chitosan group. Control group received no treatment. The muscles and skin were then sewed in layers.

Assessment and analysis

Macroscopic assessment was performed four weeks postoperatively. Six rats were randomly selected from each group and anesthetized. The surgical sites were reopened and the epidural scar adhesion underwent double blind evaluation and the results were classified based on the Rydell classification[19] (Grade 0 = epidural scar tissue was not adherent to the dura mater; Grade 1 = epidural scar tissue was adherent to the dura mater, but easily dissected; Grade 2 = epidural scar tissue was adherent to the dura mater and difficultly dissected without disrupting the dura matter; Grade 3 = epidural scar tissue was firmly adherent to the dura mater, and could not be dissected).

Hydroxyproline content analysis was performed after four weeks. Six rats were sacrificed with an overdose of pentobarbital sodium after macroscopic observation. Scar tissue of approximately 5 mg wet weight was obtained from the laminectomy sites. The content of hydroxyproline of the scar tissue from the different groups was determined. The samples were lyophilized, ground and hydrolyzed with 6mol/l HCl at 130°C for 12 h separately. Then, they were neutralized with 2.5 N NaOH on the indication of methyl red. 1 ml chloramine T was added to the hydrolyzed samples and hydroxyproline standards of four known concentrations. After incubation for 20 min at room temperature, 1 ml hydroxyproline developer (β-dimethylaminobenzaldehyde solution) was added to the samples and the standards. The solution's absorbance was measured at 558 nm with a spectrophotometer and the hydroxyproline content per milligram of scar tissue was calculated according to the standard curve constructed by the serial concentration of commercial hydroxyproline.

Six rats were selected from each group after four weeks. After anesthesia by intraperitoneal injection of pentobarbital and following intracardial perfusion with 4% paraformaldehyde, the whole spine columns including surrounding muscle tissues were removed in L1 location. The specimens were firstly decalcified by ethylenediamine tetraacetic acid (EDTA) for 30 days, then dehydrated and embedded in paraffin. Eight successive transversal sections of 4 m were made through the L1 vertebra from the top to the bottom.

Four odd sections were stained with hematoxylin-eosin and the epidural scar adhesions were evaluated using a light microscope at a magnification of 40. Three counting areas were selected in the middle and at the margins of the laminectomy sites within each section. Each counting area was approximately 100 × 100 μm. The number of fibroblasts was calculated at a magnification of 400. The number of fibroblasts in each section was defined as the mean number from three fields, and the number for each rat was defined as the mean number from the four sections.

Four even sections were stained with Masson's trichrome, and the density of collagen tissue was observed using a light microscope at a magnification of 100. Densitometric analysis of collagen tissue was also performed. The sections stained with Masson's trichrome were photographed using a light microscope (Olympus BX50, Tokyo, Japan) connected to a CCD camera (Olympus DP70, Japan). The optical density value of positively stained collagen was determined using Image Pro Plus 6.0 image analysis software.

Table I. The grades of epidural scar adhesion in rats according to Rydell's classification.

Group	Grade			
	0	1	2	3
Simvastatin	3	3	0	0
Chitosan	0	1	4	1
Control	0	0	0	6

Six rats were randomly selected from each group. The values within the table represent the number of rats.

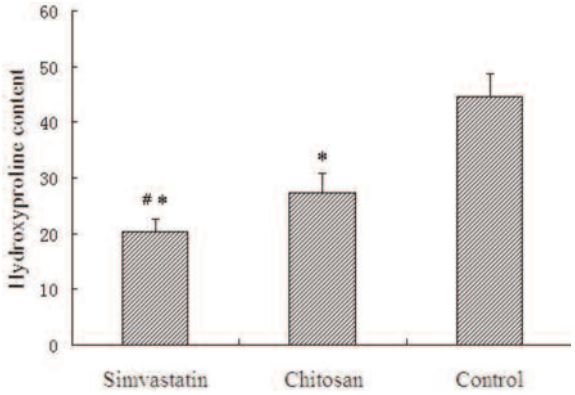

Figure 1. The hydroxyproline contents of epidural scar tissues in each group. Hydroxyproline content was expressed as μg/mg. $^{*}p < 0.05$, compared with the hydroxyproline content in control group. $^{\#}p < 0.05$, compared with the hydroxyproline content in chitosan group.

Statistiscal Analysis

Data analysis was performed by SPSS software (version 15.0). The data are shown as the mean±standard deviation. Tukey's test was used to calculate significant differences in hydroxyproline content, fibroblast number and optical density. $p < 0.05$ was taken as statistically significant in all analyses.

Results

The recovery of all rats was stable after the operations. There was no case of necrosis, neurological deficit, wound infection, or disturbance of wound healing in rats.

Macroscopic observation showed that loose fibrous scar tissues were observed in the laminectomy sites in the simvastatin group, which could be easily removed without bleeding. Moderate scar tissues were found in chitosan group. However, severe and dense epidural scar adhesions were observed around the laminectomy sites in the control group. The grades of epidural scar adhesion in rats were evaluated according to Rydell's classification (Table I).

The hydroxyproline contents of epidural scar tissues in each group were shown in Figure 1. The hydroxyproline contents of simvastatin and chitosan groups were significantly lower than that of control group ($p < 0.05$). Furthermore, the hydroxyproline contents of simvastatin group were significantly lower than those of chitosan group ($p < 0.05$).

Epidural Adhesion in Histological Analysis

In the simvastatin group, loose epidural scar tissues with no or little adhesions were observed in the laminectomy sites (Figure 2A). Little fibroblasts appeared in the laminectomy sites and were significantly less than those of the other groups. In the chitosan group, moderate epidural scar tissue adhered to the dura mater were ob-

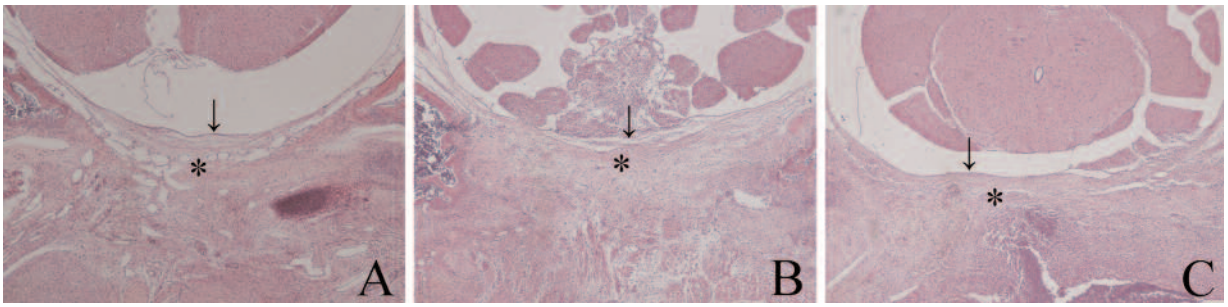

Figure 2. Representative histological images of epidural scar adhesion in the laminectomy sites of rats treated with simvastatin ***(A)***, chitosan ***(B)*** and saline ***(C)***. Loose scar tissues *(asterisk)* with no or little adherence to dura mater *(arrow)* were found in simvastatin group. The laminectomy site in chitosan group was mainly covered with moderate scar tissue *(asterisk)* with adherence to dura mater *(arrow)*. The laminectomy site in control group showed dense scar tissues *(asterisk)* with severe adherence to dura mater *(arrow)*. The sections were stained with hematoxylin-eosin and the magnification was 40.

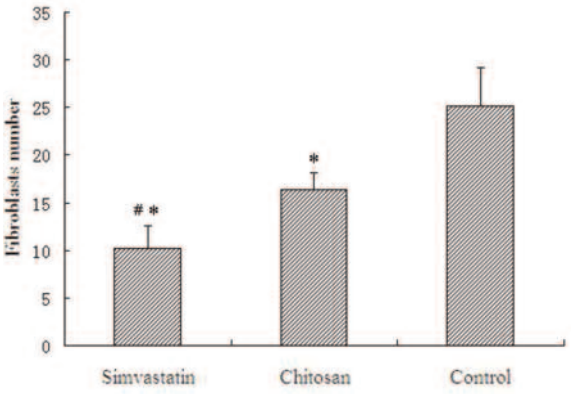

Figure 3. The fibroblast counting in epidural scar tissue in each group. Fibroblast counting was expressed as the number/counting area. $^{*}p < 0.01$ compared with the fibroblast counting of control group. $^{\#}p < 0.05$ compared with the fibroblast counting of chitosan group.

served in laminectomy sites (Figures 2B). The number of fibroblasts significantly increased in the laminectomy sites compared with that of the simvastatin group. However, severe and dense epidural scar tissues with widespread adhesions to the dura mater and dorsal muscle were observed in the laminectomy sites in the control group (Figures 2C). A large amount of fibroblasts appeared in the laminectomy sites. The number of fibroblasts in the epidural scar tissue was shown in Figure 3.

The Density of Collagen Tissue

In the simvastatin group, weak collagen tissue hyperplasia was observed in the laminectomy sites, which obviously decreased compared with those of other groups (Figure 4A). In the chitosan group, collagen tissue hyperplasia significantly increased in the laminectomy sites compared with that of the simvastatin group (Figure 4B). However, extensive and dense collagen tissue hyperplasia was observed in the laminectomy sites of the control group (Figure 4C). The densitometric analysis of the collagen tissue hyperplasia in the epidural scar tissue was shown in Figure 5.

Discussion

This study showed that topical application of simvastain could reduce epidural scar adhesion in rats after laminectomy by inhibiting fibroblast proliferation and collagen synthesis.

The formation of epidural scar adhesion following laminectomy and discectomy can result in unfavorable clinical outcomes such as radicular or low back pain. Patients with epidural scar adhesion suffer from radicular pain 3.2 times more than those without it and the possible reason is the compression of scar tissues on anatomic structures such as nerve root, which increases the sensitivity of the nerve root[20,21].

Though the formation of the epidural scar adhesion in response to injury is not well understood, it is reported that many factors contribute to epidural adhesion. It is believed that the fibroblasts play important roles in the development of epidural adhesion. In the laminectomy sites, fibroblasts propagate and produce collagen tissues to repair the local defects after being activated by inflammatory cytokines and growth factors, such as transforming growth factor-beta and basic fibroblast growth factor, which gradually form scar tissues and result in epidural adhesion[22,23]. To review the literature, there were

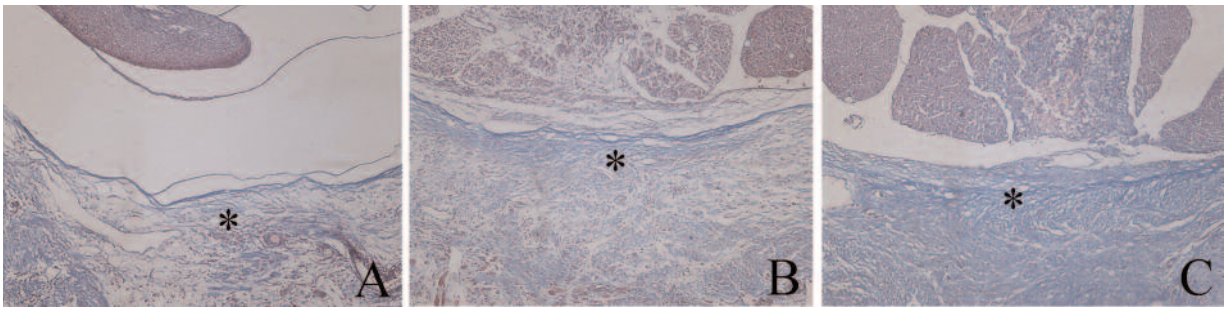

Figure 4. Representative histological images of collagen tissues hyperplasia in epidural scar tissues of rats treated with simvastatin ***(A)***, chitosan ***(B)*** and saline ***(C)***. The collagen tissues show blue in the section stained with Masson's trichrome. The density of collagen tissue *(asterisk)* in the simvastatin group was significantly less than those of chitosan group and control group. The sections were stained with Masson's trichrome and the magnification was 100.

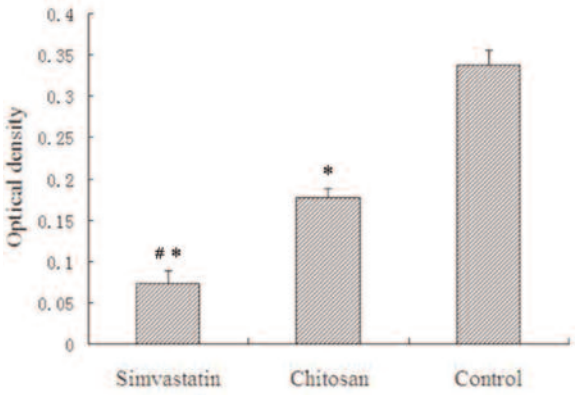

Figure 5. The optical density of collagen tissues in each group. $^{*}p < 0.01$ compared with the optical density value of the control group. $^{\#}p < 0.05$ compared with the optical density value of chitosan group.

many hypotheses and treatments for epidural scar adhesion; however, there was no effective way to solve this problem until today.

Simvastatin, one of 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase inhibitors, has been used clinically as a cholesterol-lowering drug to treat hyperlipidemia. Recently, accumulating evidences including in animal studies and clinical trials indicate that simvastatin has anti-fibrotic and anti-inflammatory effects[24,25]. It can inhibit the production of Rho-related protein and small GTPases, which suppress the immune response through inhibition of inflammatory cells and inflammatory cytokines. Moreover, some experiments have proven that simvastatin exhibited anti-fibrotic activity, not only involved in the anti-inflammation effect, but the other ways[26-28]. Previous studies also showed that simvastatin could reduce the development of pulmonary fibrosis and intraperitoneal adhesions.

In this study we found that simvastain showed significant anti-adhesion effect after laminectomy in rats. The Rydell classification, the hydroxyproline content, the number of fibroblasts and the optical density of the collagen in the simvastain group were significantly lower than those of the chitosan group and the control group, which were in accord with the results of histological analysis.

In addition to these favorable effects, simvastatin is safe for human. It can promote bone healing by inducing HIF-1 and BMP-2 expression. Another study has showed that simvastatin could repair spinal cord injury by stimulating bone marrow stromal cells to the injured area and promoting the expression of brain-derived neurotrophic factor (BDNF)[29,30]. The fracture and spinal cord injury are often the major reasons for the surgery of laminectomy and discectomy. Hence, we can obtain beneficial effects if the simvastatin is used in preventing epidural scar adhesion. In this study, we found that the topical application of simvastatin could reduce epidural scar adhesion in rats. However, we only evaluate the effect of simvastatin on reducing scar adhesion by histological examination. Further research is necessary to determine the optimal dosage, safety and the more effective vehicle of simvastatin.

Conclusions

The topical application of simvastain has a valid therapeutic effect on reducing epidural scar adhesion after laminectomy in rats by inhibiting fibroblast proliferation and collagen synthesis. It indicates that simvastain may be a potentially effective drug to prevent epidural adhesion after lumbar laminectomy.

Acknowledgements

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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