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Prevention effect of medical self-crosslinking sodium hyaluronate gel on epidural scar adhesion after laminectomy

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ABSTRACT

Objective: To analyze the effect and medical self-crosslinking sodium hyaluronate gel on epidural scar adhesion after laminectomy. **Methods:** A total of 24 New Zealand L5 laminectomy rabbits were randomly divided into four groups, group A as the control group without any treatment; group B covered by sodium hyaluronate gel; group C and group D covered by 0.5 and 1.0 mL medical self-crosslinking sodium hyaluronate gel. All rabbits were scored at various time points after 2, 4, 6, 8, 12 weeks, then the formation of scar was observed. **Results:** In Groups B, C, D loose scar tissue occurred after 2 weeks of the operation, scar tissues were significantly less than that in group A, with mild inflammatory reaction. After 8 weeks, the scar tissues of group B were significantly more than that of groups C, D. After two weeks, group B, C were back to the preoperative level; After 4 weeks, group D was back to the preoperative level; After four weeks, the CSEP of group A was increased significantly, which was significantly higher than that in groups B, C, D. The motor function score of group A, group B and group C were the same as preoperative, but that in group D it was decreased significantly, and then it gradually recovered. After 4 weeks it kept a stable level. The motor function score of group A was decreased gradually after the operation, which kept a stable level after 4 weeks, the motor function score was significantly lower than that in groups B, C, D. **Conclusions:** Determination of somatosensory evoked potentials is sensitive indicator of spinal cord injury; Application of medical self-crosslinking sodium hyaluronate gel is effective on epidural scar adhesion after laminectomy.

1. Introduction

Laminectomy is one of the most common surgical procedures of spine surgery. Due to the formation of a large amount of scar tissue postoperative laminectomy defect often severely affects the outcome of spinal surgery. Special materials covering the spinal dural and prevention of postoperative adhesions become a research hotspot. Currently semi-fluid biomaterial hyaluronic acid, chitosan, biofilm or bone tissues were adopted, each of them has its own characteristics, but there still are defects. Because self-crosslinking sodium hyaluronate can maintain the physical and chemical properties of the original hyaluronic acid clumps and extend degradation time *in vivo*, it has been used in general surgery and gynecology for anti-adhesion[1-3]. In this study, the application of medical self-crosslinking sodium hyaluronate gel in treatment of epidural scar adhesion after laminectomy is explored.

2. Materials and methods

2.1. Experimental animals and agents

24 adult New Zealand white rabbits, aged 4 to 5 months were selected, which were provided by the Institute of Experimental Animal Center of our hospital. All rabbits had no spine and disc disease confirmed by MRI. Medical sodium hyaluronate gel and self-crosslinking sodium hyaluronate were purchased from Hangzhou Xiehe Medical Supplies Ltd., kept at room temperature avoid light.

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2.2. Animal model establishment and grouping

After instrument sterilization, they were anesthetized with 10% chloral hydrate 5 mL/kg by ear vein injection, lumbosacral back hair was cut, and then the hair root was removed with 8% sodium sulfide. The rabbits were fixed on the operating table at prone position, and lumbosacral was padded. The electrodes were pierced into subcutaneous of both hind limbs malleolus, parietal, midpoint of eyes, cheeks respectively. The first electrical stimulation was carried out, the latencies values of preoperative somatosensory evoked potentials (CSEP) was recorded. After sterilization, the skin and fascia was cut in 3 cm, blunt dissection was performed layer by layer, L5 lamina spinal dural was removed to expose area. Second electrical stimulation was carried out, the latencies values CSEP after laminectomy was recorded. Unusual CSEP rabbit was excluded and the new rabbit model was supplemented.

24 rabbits were randomly divided into four groups, group A (control group) injected with saline 0.5 mL, group B filled with sodium hyaluronate gel 0.5 mL, group C filled with medical self-crosslinking sodium hyaluronate gel 0.5 mL, group D filled with medical self-crosslinking sodium hyaluronate gel 1 mL. After filled up the incision was sutured layer by layer, gentamicin 80 000 units were given intramuscularly postoperative to prevent infection. The third electrical stimulation was Carried out before awake, CSEP latencies values was recorded.

2.3. Indicators observation

2.3.1. Determination of somatosensory evoked potentials

The electrodes were pierced into subcutaneous of both hind limbs malleolus, parietal, midpoint of eyes, cheeks, respectively. Parameters were as follows: frequency 2.7 Hz, duration of 200 ms, filtered signal frequency: 2-2 000 Hz. the CSEP latencies values was measured and recorded 2, 4, 6, 8, 12 weeks preoperative, intraoperative, postoperative respectively.

2.3.2. Scored motion of hindlimb

Tarlov was used to measure rabbit hindlimb motor function 1 day preoperative and postoperative and 8 weeks after surgery. The standard was completely paralyzed limbs 0 points; Hindlimb could horizontally paddle was 1 point; Hindlimb could be moved but not weight-bearing 2 points; hindlimb could weight and walk but unstable 3 points; normal function 4 points.

2.3.3. General observation

Complete segmental spinal specimens were removed from one animal of each group1 day and 2, 4, 6, 8, 12 week postoperatively, the epidural adhesion was observed. Refer to Rydell scoring criteria: no subdural and scar tissue adhesions was 1 point; Only a small membrane adhesion between the scar tissue and dura mater, easy to separate was 2 points; Dural and scar adhesions range less than 2/3 of aminectomy defect diameter was 3 points; Extensive and dense adhesions between the scar tissue and dura mater, could not blunt dissection, the range was greater than 2/3 of aminectomy defect diameter or scar tissue invaded into the nerve root was 4 points.

2.4. Statistical analysis

The data was analyzed with SPSS 13.0 software. Data were expressed as mean±SD values, and compared with single factor analysis of variance. Comparison between the two groups was carried out by SNK test method. $P<0.05$ was regarded as statistically significant difference.

3. Result

3.1. General observation

Animals could be awake generally within about 40 min postoperatively, self foraging and drinking. All rabbits showed no incision infection. After two weeks, group A showed significantly scar tissue and obvious inflammatory response until 6 weeks, after 6 weeks the scar tissue remained the same, without change. Groups B, C, D had the formation of loose scar tissue after 2 weeks of the operation, scar tissues were significantly less than that in group A, with mild inflammatory reaction. After 6 weeks groups C and D were basically the same postoperatively. After 8 weeks, the scar tissues of group B gradually increased, which were significantly more than that of groups C, D. The difference was statistically significant ($P<0.05$) (Table 1).

3.2. Somatosensory evoked potentials

CSEP in group D after anesthetized was significantly longer, which were slightly extended in the other groups. After 2 weeks, group B, C were back to the preoperative level; After 4 weeks, group D was back to the preoperative level; After4 weeks, the CSEP of group A increased significantly, which was basically the same 6 weeks postoperatively, significantly higher than that in groups B, C, D. CSEP latency of each time period in each group showed no significant differences ($P>0.05$) (Table 2).

3.3. Motor function score

The motor function score of group A ,group B and group C were the same with preoperative, but that in group D it was decreased significantly, the difference was statistically significant, and then it was gradually recovered. After 4 weeks it kept a stable level. The motor function score of group A was decreased gradually after the operation, which was stable after 4 weeks, the motor function score was significantly lower than that in groups B, C, D, and the difference was statistically significant ($P>0.05$) (Table 3).

Table 1
Score results of the formation of scar tissue.

Groups	Preoperative	1d postoperatively	2 weeks	4 weeks	6 weeks	8 weeks	12 weeks
Group A	1	1	2.8±0.6	3.2±0.6	3.5±0.8	3.6±0.8	3.6±0.8
Group B	1	1	1.4±0.4*	1.8±0.4*	2.3±0.6*	2.8±0.7*	3.0±0.8*
Group C	1	1	1.4±0.3*	1.7±0.4*	2.0±0.5*	2.0±0.5*△	2.0±0.5*△
Group D	1	1	1.4±0.3*	1.7±0.3*	2.0±0.4*	2.1±0.5*△	2.1±0.5*△

Note: Compared with group A *$P<0.05$; compared with group B △$P<0.05$.

Table 2
CSEP results of each group.

Groups	Preoperative	1d postoperatively	2 weeks	4 weeks	6 weeks	8 weeks	12 weeks
Group A	20.8±0.7	21.5±0.8	22.4±0.7	23.4±0.7	24.5±0.7	24.4±0.6	24.4±0.7
Group B	20.7±0.5	21.4±0.9	20.5±0.7	20.6±0.7*	20.6±0.7*	20.7±0.8*	20.6±0.8*
Group C	20.9±0.5	21.5±0.7	20.7±0.8	20.6±0.8*	20.6±0.6*	20.5±0.7*	20.6±0.6*
Group D	20.8±0.6	23.5±0.7*△	21.6±0.8	20.5±0.6*	20.6±0.8*	20.7±0.5*	20.5±0.6*

Note: Compared with group A *$P<0.05$; compared with group B △$P<0.05$.

Table 3
Motor function score of each groups.

Groups	Preoperative	1d postoperatively	2 weeks	4 weeks	6 weeks	8 weeks	12 weeks
Group A	4	4	3.4±0.2	3.0±0.5	3.0±0.6	3.0±0.4	3.0±0.4
Group B	4	4	4*	4*	4*	4*	4*
Group C	4	4	4*	4*	4*	4*	4*
Group D	4	3.2±0.2*△	3.4±0.2△	3.9±0.1*	3.9±0.1*	4*	4*

Note: Compared with group A *$P<0.05$; compared with group B △$P<0.05$.

4. Discussion

Epidural adhesion is one of the important causes of the failure laminectomy. Tissue repair is accomplished by fibrous tissue, early granulation and late scar tissue formation. Previous studies suggest that fibroblasts usually appear on 2d postoperatively, spread along with hematoma and ingrowth of blood clots together with capillary, which can stimulate cell proliferation by the chemotactic effect of inflammatory mediators and growth factors, eventually fibroblast aggregated, collagen synthesis, partial scar formated and affect the prognosis[4-7]. Currently there is no effective means to prevent the body's process of fibrosis, we can only use biological materials physically blocking epidural scar fibrosis[8].

Most studied and clinically appliedepidural adhesion prevention materials were mainly the high molecular weight sodium hyaluronate, because it has good hydrophilicity and low immunogenicity, which is a material widely used for the prevention of tissue adhesion after various surgery. Due to rapid degradation traditional hyaluronic acid failed to fill in the epidural surface for a long time, so the long-term prevention adhesions is not ideal[9-13]. Therefore, in order to overcome the long-term effects, this study used medical self-crosslinking sodium hyaluronate gel. It has good hydrophilicity and low immunogenicity, but also difficult to degrade and can present in the epidural surface for a long time, which become an ideal experimental material.

CSEP as a sensitive indicator of spinal cord injury can ensure the objectivity and accuracy of the experimental data[14]. This study suggests that CSEP in group D after anesthetized was significantly longer, which were slightly extended in the other groups. After 2 weeks, group B, C were back to the preoperative level; After 4 weeks, group D was back to the preoperative level; After 4 weeks, the CSEP of group A increased significantly, which was basically the same 6 weeks postoperatively, significantly higher than that in groups B, C, D[15]. Therefore mild irritation can cause significant changes of CSEP and the implantation of polymer material has a significant anti-adhesion effect at the early stage. We believed that the sodium hyaluronate macromolecular network structure can combine a large amount of water to form a physical barrier and regulate cell function and so on. Surrounding the dura and nerve roots can effectively prevent the occurrence of adhesions, especially two weeks before severe inflammation. It is an excellent material to prevent spinal adhesions and also provide normal space for the dural sac and nerve root, maintain normal blood circulation and cerebrospinal fluid circulation[16-20]. But group D had an abnormal CSEP, but gradually returned to normal with time, considered the excessive injection of polymer materials may lead to spinal cord compression and causing changes in CSEP. With the degradation of macromolecules, the spinal cord oppression can gradually disappear and CSEP also returned to normal[21].

The tissue losses of group A cause obviously inflammation. A lot of fibroblast cells can synthesize collagen fibers and lead to local significant fibrosis, and scar fibrosis also

cause the spinal cord persistent oppression. This study Tarlov score showed the motor function score of group A, group B and group C were the same with preoperative, but that in group D it was decreased significantly, and then gradually recovered. After 4 weeks it keeps a stable level[22]. The motor function score of group A decreased gradually after the operation, which keeps a stable level after 4 weeks, the motor function score was significantly lower than that in groups B, C, D. The motor function score is similar with the CSEP[23]. After 2 weeks, group A showed significantly scar tissue and obvious inflammatory response until 6 weeks, after 6 weeks the scar tissue remained the same, without change within the observation time. Groups B, C, D showed the formation of loose scar tissue after 2 weeks of the operation, scar tissues were significantly less than that in group A, with mild inflammatory reaction. After 6 weeks groups C and D were basically the same postoperatively. After 8 weeks, the scar tissues of group B gradually increased, which were significantly more than that of groups C, D. Therefore, medical self-crosslinking sodium hyaluronate gel has obvious advantages in preventing scar formation.

In summary, a small dose of medical self-crosslinking sodium hyaluronate gel is effective on epidural scar adhesion after laminectomy, which can provide a theoretical basis for clinical application.

Conflict of interest statement

We declare that we have no conflict of interest.

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