Experimental research on the effect of mitomycin C fibrin gel on spinal peridural scar tissue in rats

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Abstract: This paper aimed to study the best working concentration of mitomycin C (MMC) fibrin gel on spinal peridural scar tissue in rats and the sustained release function of peridural adhesion after laminectomy of rats. 96 SD rats were divided into four groups. They were conducted L1 laminectomy and sprayed FG-MMC. The concentration of MMC was detected and the best working concentration of MMC was selected. Then other 48 SD rats were divided into four groups to construct L1 vertebral plate excision model. Materials were drawn 4 weeks after the operation for Rydell grading. In addition, we observed HE staining and fibroblast proliferation and collagen distribution situation after Masson staining. And concentration of hydroxyproline was also detected. The best working concentration of MMC experiment showed that except experimental group C, the other groups all appeared drug release peak in the 4th week after the operation. MMC concentration of group C appeared drug release peak in the 5th week besides the 2nd week. Moreover, adhesion of endorhachis and peripheral tissue in group A was the most obvious while the group C was the weakest. Experiment on the slow-release effect of different adhesion materials on peridural adhesion showed that compared to the contrast group and group F, Rydell grading in group E and G was Level 0 and with low adhesion degree, little fibroblast and fibrocyte, low collagen content, regular collagen fibers arrangement and no inflammatory cells after HE staning and Masson staining. In addition, content of hydroxyproline (HOP) decreased significantly especially the group of FG-MMC mixture. It was concluded that MMC fibrin gel mixture had a good slow-release effect on adhesion of spinal peridural scar tissue in rats and the best working concentration was 0.5 mg FG-MMC/ml.

Keywords: Mitomycin C, Protein fiber gel, Scar tissue adhesion, Drug sustained release

INTRODUCTION

Laminectomy is one of the most common operation methods in spine surgery. However, the formation of endorhachis scar is one of the main causes leading to recurrence of lumbocrural pain (Lei, 2013). Since the initial stage of 70's, many scholars at home and abroad conduce research on the formation and prevention of peridural fibrous scar after laminectomy. When probing into the relationship of peridural peripheral fibrosis and failed back surgery syndrome (FBSS) after operation of protrusion of lumbar intervertebral disc, some scholars' research (Yaochuan, 2011; Lei *et al.*, 2013, Einhaus *et al.*, 1997; Zhidong and Bangchun, 2012) supported the thought in the research theory of Einhaus *et al* (1997) that fibrosis was the normal existence and the real reason that cause the symptom was not clear. However, Quist *et al* through MRI proved that peridural peripheral fibrosis and recurrent pain was directly related to FBSS (Chengliang, 2011; Quist *et al.*, 1998). In addition, the experiment of Nakano *et al* showed that postoperative inflammation was closely related to early postoperative low back pain and lower limb radiation pain (Lei, 2013; Nakano *et al.*, 1998). So far, the adhesion prevention materials that have been studied have categories of biological materials, artificial material, composite, autologous tissue, adhesion prevention drug and controlled release carrier. Several drugs have achieved good curative effect in clinical. After 1960's, slow release and controlled release preparations drew more and more attention since they have the effect of improving the accuracy, effectiveness and safety of drugs as well as decreasing adverse drug reaction and production cost (Ledao, 2011; Yi, 2014). Application of slow-release system carrier of new drug to prevent adhesion of spinal peridural fibrosis and adhesion after laminectomy has become the current research hotspot (Lei, 2013). Therefore, this paper observed the adhesion prevention effect of different medication administration concentrations and means on spinal peridural scar tissue in rats and explored the slow-release effect of FG-MMC by constructing vertebral plate.

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MATERIALS AND METHODS

Experimental animals, main material and drugs

Experimental animals: healthy adult male SD rats (Sprague Dawley rats) 280±20g. Main material and drug: MMC (Zhejiang Haizheng Pharmaceutical Co., Ltd.), PBS (Phosphate Buffered saline) buffer solution, fibrous protein (Guangzhou Beixiu Biotechnology Co., Ltd., trade name: fibrin glue), etc.

Manufacture of L1 laminectomy model

10% chloral hydrate (3 ml/kg) was injected into enterocoelia of SD rat that was fixed in prone position for anesthesia. Incision in the middle of rat spine in length of

2 cm was taken. L1 and L2 vertebral plate was visible and the whole L1 vertebral plate was removed to form 0.5cm×0.3cm defect. Endorhachis was visible. And the incision was stopped bleeding by compression. At last, L1 vertebral plate excision model was prepared.

Establishment of experimental group

Experiment of the best working concentration of MMC fibrin gel: 96 SD rats were grouped into 4 groups. They were conducted L1 laminectomy, exposed endorhachis and sprayed FG-MMC. Group A, B, C and D was corresponding to different concentration of MMC concentration (0.1, 0.3, 0.5 and 0.7 mg/ml). After the operation, the rats were placed in different cages and feed on different drugs. The feeding and management condition of all rats before and after operation were consistent.

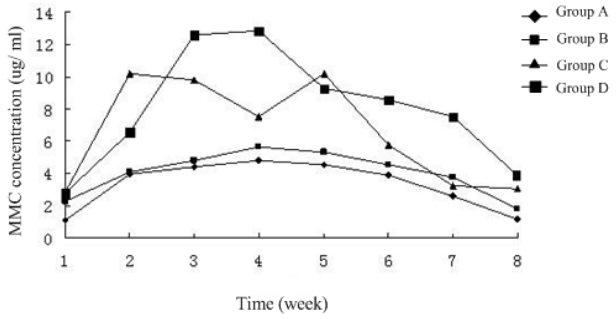

Fig. 1: Change of MMC concentration at different time points

Experiment on slow- release effect of different adhesion materials to peridural adhesion: 48 SD rats were divided into 4 groups. They were conducted L1 laminectomy and exposed endorhachis. Group E, F, G and H was corresponding to 0.5 mg/ml MMC, FG-MMC mixture of 0.5 ml FG and MMC with concentration of 0.5 mg/ml and normal saline for experiment. The postoperative rats were placed into different cages and feed on different drugs. The feeding and management condition of all rats before and after operation were consistent.

MMC standard curve drawing

PH7.4 PBS buffer solution was used to dissolve MMC into a series of standard solution with concentration of 100, 50, 25, 12.5, 6.25 and 1ug/ml. They were detected for ultraviolet spectrophotometric value in 365 nm. We fitted the standard curve and confirmed standard curve equation and coefficient.

Tissue observation index

Union status of rat wound; postoperative peridural adhesion; HOP concentration and collagen content in scar tissue; adhesion degree of dura mater and tissue; fibrocyte proliferation and collagen distribution after HE and Masson staining.

Data analysis

SPSS16.0 statistical software package was applied to draw the curve of relationship between drug release concentration of FG-MMC mixture and time (the experiment was repeated for three times). And SPSS16.0 software was also used to make statistical analysis on the obtained data. Every group of data was expressed as $\overline{X} \pm s$. Comparison between groups was adopted one-way analysis of variance and q test. The test standard was 0.05. $p < 0.05$ had statistical meaning.

RESULTS

Experiment result of the rest working concentration of MMC fibrin gel

The adhesion situation of dura meter and peripheral dura mater were observed. As to the adhesion degree, we found that group A was the most obvious because the dura mater and scar tissue could not peeled completely and group C was the weakest. Group B and C had part adhesion and the dura mater maintained completely after separation.

After operation, MMC concentration of all groups overall showed a trend of rising first and decreasing afterwards. Group had little difference with the other groups. Group A, B and C all reached the maximum MMC concentration 4 weeks after operation. The concentration was 4.4041, 5.6644, 12.8652ug/ml. Group C appeared release peak in the 2nd week with a concentration of 10.2074ug/ml. Afterwards, group C appeared the other release peak in the 5th peak with a concentration of 10.1555ug/ml. It is as shown in table 1.

The curve chart was drawn according to the change of postoperative MMC concentration at different time points. It is as shown in fig. 1.

Experimental result of slow-release effect of different adhesion materials on peridural adhesion

6 rats in every group were took and cut the operation part spine for the observation of peridural adhesion. We saw that peridural scar tissue and endorhachis had no adhesion on the part after laminectomy in MMC group. In the separation process, bleeding was less. In group of FG and group of FG-MMC, scar tissue proliferation was less, dura mater and peripheral tissue had potential gap. In the separation process, bleeding was less. Group of normal saline appeared serious adhesion. In the process of dura meter stripping, bleeding was more and the dura mate could not be exposed clearly and completely. They were done adhesion degree grading according to Rydell grading standard. It is as shown in table 2:

Rats in groups showed different histomorphology performances through HE staining after operation, as shown in table 3 and fig. 2.

Table 1: Postoperative MMC concentration at different time points

Time (week)	MMC (ug/ml)			
	Group A	Group B	Group C	Group D
1	1.07334	2.2808	2.8733	2.8124
2	3.9488	4.0796	10.2074	6.5374
3	4.4041	4.8264	9.7583	12.5672
4	4.7641	5.6644	7.5443	12.8652
5	4.5281	5.3394	10.1555	9.2553
6	3.8941	4.5212	5.7442	8.5794
7	2.6032	3.7742	3.2161	7.4893
8	1.1924	1.8123	3.0567	3.8993

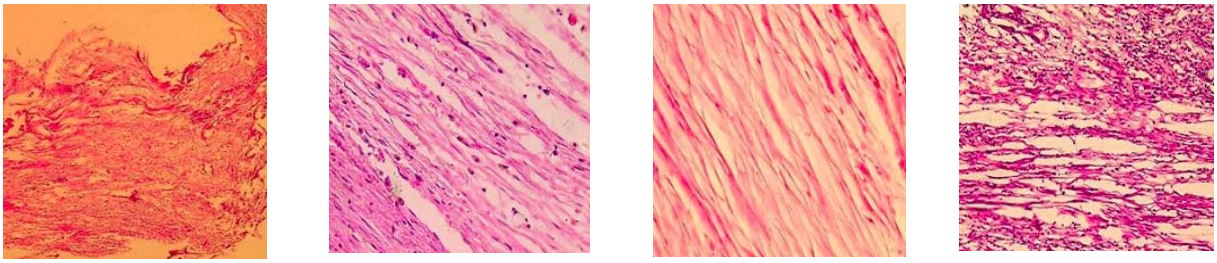

Fig. 2: Histomorphology of group E, F, G and H after HE staining

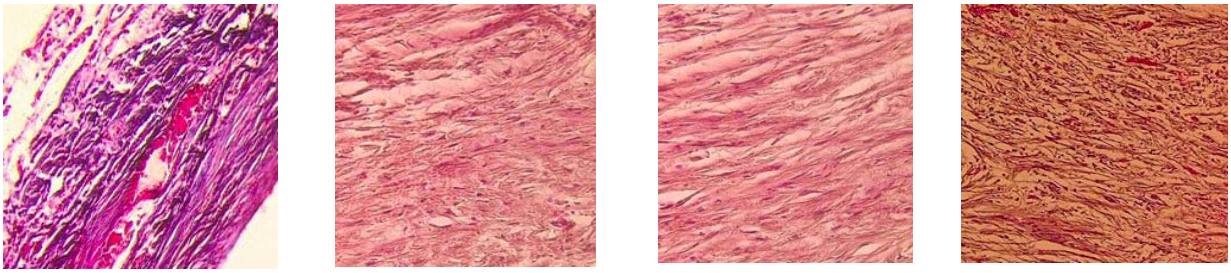

Fig. 3: Histomorphology of group E, F, G and H after Masson staining

The rats in groups showed different histomorphology performance after Masson staining, as shown in table 4 and fig. 3

Concentration of HOP was detected and calculated in peridural scar tissue of rats 4 weeks after operation. And collagen content was also speculated. We found that the concent in group E and F significantly decreased, especially the group FG-MMC, as shown in table 5

DISCUSSION

The nature of peridural scar adhesion was to repair local damage caused by laminectomy by fibrous connective tissue hyperplasia (Hailei *et al.*, 2011). MMC can inhibit the conversion of fibroblast into proliferation phenotype so that restrain the proliferation of fibroblast (Lei, 2013). So far, the absorbable and nonabsorbable biological membrane in clinical are used to prevent adhesion after spine surgery. FG-MMC mixture in this experiment was absorbable material. And the physical barrier function of FG and slow-release effect of MMC were applied to inhibit the generation of fibrocyte and extra cellular matrix so that prevent adhesion. In the experiment, the other groups all appeared drug release peak 4 weeks after the operation except group C. And the concentration of group reached a release peak of 10.2074ug/ml in the 2nd week and 10.1555ug/ml in the 5th week. In the rest of time, it released in a relative low concentration and gradually decreased. That illustrated that FG-MMC had certain slow release function, which provided basis for the following experiment.

Table 2: Rydell grading of postoperative peridural adhesion (n=6)

Group	Grade			
	0	1	2	3
Group E	4	2	0	0
Group F	0	4	2	0
Group G	5	1	0	0
Group H	0	0	0	6

There are three methods for preventing the adhesion of dura mater and nerve root so far: ① minimally invasive surgery; ② drug application such as hormone,

Table 3: Postoperative histomorphology performance after HE staining

Group	Adhesion situation	Fibroblast and fibrocyte	Inflammatory cells
Group E	No obvious adhesion	A small number of mixing	None
Group F	Close adhesion	Much mixing	Few
Group G	No obvious adhesion	Little mixing	None
Group H	Close adhesion	A large number of mixing	Few

Table 4: Postoperative histomorphology performance after Masson staining

Group	Peridural scar tissue situation	Collagen tissue proliferation	Collagen content	Arrangement of collagenous fiber
Group E	Loose	Unobvious proliferation	Little	Regular
Group F	Compact	Obvious proliferation	Much	Irregular
Group G	Loose	Unobvious proliferation	Little	Regular
Group H	Compact	Obvious proliferation	Much	Irregular

Table 5: HOP content of rats in four groups

Group	Numbers of animals	ug/ml wet tissue HOP content ($\overline{X} \pm s$)
Group E	6	18.16±3.14
Group F	6	27.47±2.82
Group G	6	16.81±2.31
Group H	6	38.15±2.92

chemotherapeutics, etc (Zhiying *et al.*, 2010; Jue *et al.*, 2013); ③ application of contact of peridural biofilm and scar tissue (Xiaolei *et al.*, 2012; Cong *et al.*, 2013). MMC can be used for the formation of scar. A good antis ticking effect can be achieved when MMC is wet compressed outside the dura mater. However, potential toxic and side effect exist. Therefore, we should slowly control its release. Fibrin gel has advantage of rapid degradation besides physical barrier function. Similar to cytomembrane, it is good to reduce adhesion degree and a kind of promising slow-release material (Lei, 2013). The combination of FG and MMC in the research had a more effective effect on preventing the adhesion of dura mater and peripheral tissue after spine surgery compared to the single application of FG and MMC. Meanwhile, based on the slow-release effect of FG, MMC had a small stimuli on the nerve of rats and had no obvious side effect on tissue healing. Group FG-MMC and group MMC had the minimum adhesion degree. Compared to group MMC, FG-MMC can be used in operative region quantification ally. Single application of MMC is not easy to control and the safety is hard to ensured (Lei, 2013; Shenghua *et al.*, 2011).

FG-MMC gel delivery system combines two mechanism of physical barrier and inhibiting fibroblast. It releases small dose of MMC when reducing the content of peridural scar tissue. Compared to single use, it can achieve the purpose of toxicity reducing and efficiency enhancing and have a good application prospect in peridural scar tissue adhesion (Lei, 2013).

CONCLUSION

In conclusion, mitomycin C fibrin gel mixture integrates the slow release function of single application of mitomycin C with good hemostasis and barrier functions of single application of fibrin gel. In addition, it remits the toxic and side effects of mitomycin C such as potential stimulation, the effect on gastrointestinal tract, wound healing, etc. It has a good effect on the slow release of adhesion in spinal epidural scar tissue and the best working concentration is 0.5mg FG-MMC/ml.

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