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Controlled-Release SHH Fibrin Scaffold Transplantation Promotes Function Recovery of the Spinal Cord Injury in Rats

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ABSTRACT

Objective: investigate that the transplantation of sonic hedgehog (SHH) fibrin scaffold promotes recovery of the spinal cord injury in rats. **Method:** first, the model of controlled-release SHH fibrin scaffold was made in vitro as the experimental group and observe the controlled-release performance. Second, 60 healthy SD rats were assigned to prepare models of complete transection of spinal cord, divided into 3 groups: SCI group (simple transection of spinal cord), FG group (fibrin group), F-SHH group (sonic hedgehog-fibrin scaffold transplantation group). Grade hind limbs (BBB) of rats every week. The spinal cord segments were got out in 3 months after operation and went through immunohistochemistry and immunoblotting detection. Observe the expression of NF200, GAP43 and GFAP. **Result:** (1) SHH Fibrin showed a good effect of slow release. (2) F-SHH group showed a more significant improvement in BBB score that presented a rising trend in the whole, compared with the other two groups and the difference is statistically significant ($P < 0.05$). (3) The relative amounts of NF200 and GAP43 in F-SHH group were much higher than those in the other two groups, while the relative amount of GFAP was lower and the difference is statistically significant ($P < 0.05$). **Conclusion:** Controlled-release SHH fibrin scaffold transplantation will effectively recover complete spinal cord transection of rats.

Introduction

At present, recovering spinal cord injury with scaffold transplantation is the tissue engineering possessing good application prospect. In ideal conditions, the tissue engineering scaffold will be well compatible with spinal cord, and the fibrin scaffold will be converted into neuronal tissue with a slow effect of spinal cells to play an important role in promoting regeneration of nerve fiber[1]. Major material used for constructing scaffold at this stage is fibrin, the extracellular matrix existing in blood, which will realize effective recovery of spinal cord injury as the carrier of growth factor controlled-release system and cell transplantation[2]. This paper prepared various models with 60 healthy SD rats and performed a series of treatment to observe how SHH fibrin scaffold transplantation promoted

the recovery of spinal cord injury in rats.

1 Data and Method

1.1 Common Data

60 SD adult healthy female rats, in the weight between 231 g and 252 g, averaged to be (240.58 ± 8.32)g, all come from the laboratory animal center of the university where the author is working. Other items or instruments are as follows: (1) Fibrinogen; (2) Thrombin; (3) Anhydrous calcium chloride; (4) SHH; (5) Anti-mouse NF200, anti-rabbit GAP43, anti-rabbit GFAP; (6) Kit used in ELISA method.

1.2 Method

1.2.1 Prepare the Model of Controlled-Release SHH Fibrin Scaffold in Vitro

First, dissolve fibrin monomer powder into normal saline and put it into 37℃ thermostat for the purpose of accelerating dissolution rate to prepare fibrin solution, of which the density shall be 40 mg / mL. Then dissolve thrombin powder into calcium chloride solution (40 mmol / L), of which the density shall be 0.02 mg / mL. In the end, fully dissolve 0.025 mg SHH powder into 0.25 mL thrombin solution to prepare the 100 ng / μL SHH solution, of which take 10 μL out to mix with 40 μL thrombin solution, followed by mixing with 50 μL original fibrin solution such that they have reaction at the bottom of centrifuge tube to produce gel that is the SHH fibrin scaffold.

1.2.2 Group Animals and Establish Models of Spinal Cord Injury

Divided the 60 adult female SD rats into 3 groups that is F-SHH group, FG group and SCI group to go through the following three steps respectively: (1) Transplant controlled-release SHH fibrin scaffold into injured spinal cord; (2) Transplant fibrin glue into injured spinal cord; (3) None of transplantation is done but complete spinal cord transection. The method to transect spinal cord is as follows: use 30 mg / kg pentobarbital sodium for intraperitoneal anesthesia. Shave for skin preparation, fix the rat on the bench in prone position, position the vertebral spinous process at T12 section, make an incision in the center of back in germ-free conditions, separate skin and subcutaneous fascia to expose spinous processes and vertebral plates at T9-L1 sections, clamp spinous processes and vertebral plates at T9-12 sections with a hemostat to transect the spinal cord at T10-11 sections and leave a 2 mm gap.

1.3 Observation Indicator

1) Grade BBB score for the three groups of rats every week in 3 months after operation. Before grading, make the rats get used to the opening environment, after which guide them to keep walking and grade their hind limbs average the scores.

2) Conduct histological observation. Open the chest for rapid cardiac perfusion, replace the blood in the rat and fix it with paraformaldehyde, and then take out spinal cord tissue in and on the two layers around the center of transection, fix it with formaldehyde for 24 h before paraffin section and develop immunohistochemistry and determine the relative amount of GFAP, NF200 and GAP43[3].

3) Obtain fresh spinal cord specimens. Add lysate to cleavage on ice for 45 min, break the tissue with ultrasound, put it into the buffer to boil before centrifugation, and then take the supernatant to keep in -80℃ environment for the use in immunoblotting assays[4].

1.4 Statistical Analysis

Verify clinical data of patients participating the experiment with statistical software package SPSS17.0. BBB scores are required to be compared among groups and analyzed with one-way variance while GFAP, NF200 and GAP43 are expressed with mean values. The comparison among groups is also analyzed with one-way variance. If the statistic $P < 0.05$, it means the result is statistically significant.

2 Result

2.1 Controlled-Release Effect of SHH Fibrin

In figure 1, the result of one-way variance analysis between the density of SHH sample and absorbance is $R^2 = 0.996$, approaching 1, which means high fitting degree and high reliability that is good controlled-release effect of SHH fibrin.

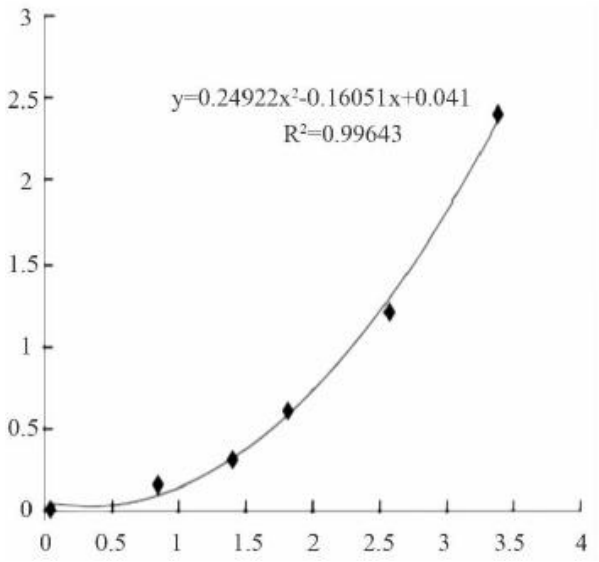

Figure 1 Controlled-Release effect of SHH fibrin (the vertical axis means density and the horizontal axis means absorbance)

2.2 BBB Score

As shown in figure 2, F-SHH group showed a more significant improvement in BBB score that presented a rising trend in the whole, compared with the other two groups and the difference is statistically significant ($P < 0.05$).

2.3 Relative Content

As shown in figure 3, the relative amounts of NF200 and GAP43 in F-SHH group were much higher than those in the other two groups, while the relative amount of GFAP was lower and the difference is statistically significant ($P < 0.05$).

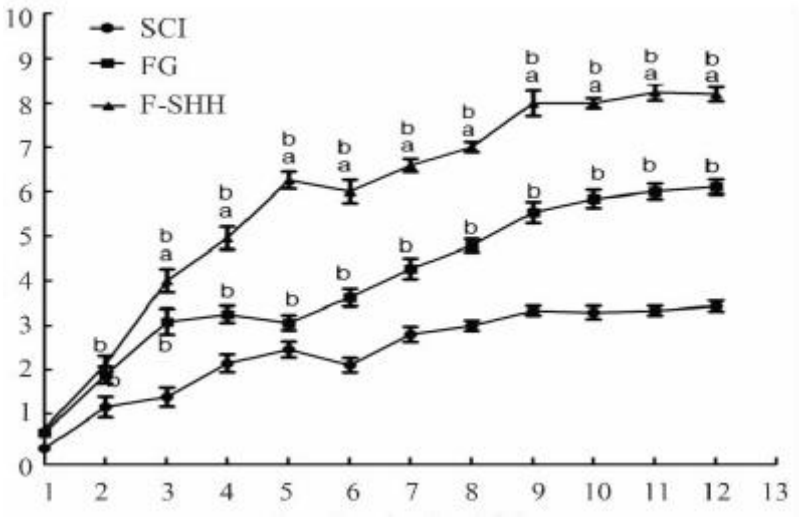

Figure 2 BBB scores trend of three groups of animal models (the vertical axis means BBB score and the horizontal axis means time)

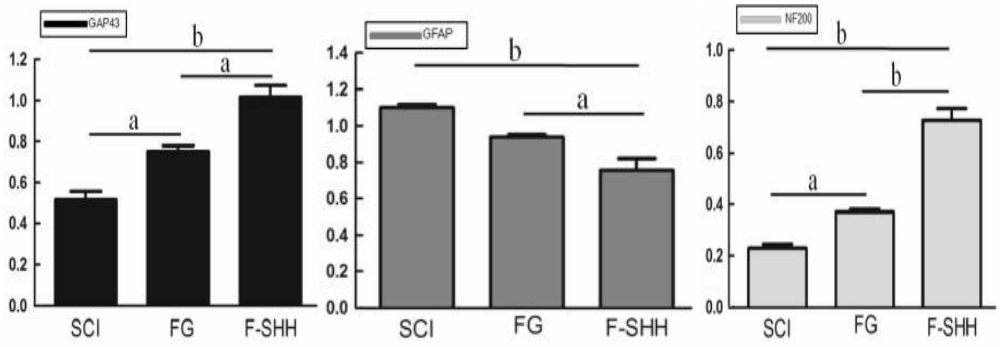

Figure 3 Relative amounts of GFAP, NF200 and GAP43 (the vertical axis means relative amount)

3 Discussion

Spinal cord injury is a serious injury for the central nervous system. Most patients will suffer severe consequence such as acroparalysis or even various complications, which will not only increase the medical cost, but also bring huge burden to the patient's family. However, fibrin as an extracellular matrix will turn to biological adhesive molecular material that is fibrin glue with high biodegradability and plasticity affected by calcium chloride and thrombin. It is discovered from clinical practice that fibrin glue has achieve satisfied result in the treatment of spinal cord injury[5-6]. On this basis, people are trying sonic hedgehog, one of the five hedgehogs to repair injured spinal cord. It is known from existing research products that the neural tube structure at back and belly side produced by SHH will act on neural epithelium to maintain its activity to promote the generation of neural tube. For nervous system, SHH is able to promote its development and differentiation and have a certain regulating effect on it[7]. Cui Xuewen also points out in his research that SHH is able to promote the formation of neuron synapse and has a significant promoting effect on neuron survival and neurite outgrowth, besides, it is also to turn fibrin glue to its controlled-release carrier and promote the formation of myelin sheath as well. If SHH is injected into fibrin glue, it will construct a controlled-release SHH fibrin scaffold, and if transplant it to the injured spinal cord in rats, it will reconstruct the spinal cord tissue to result in hyperplasia of keratin scar [8-10]. The result does verify the opinion above. It is known from Fig. 1 that SHH fibrin has a good effect to slow release; the BBB score of F-SHH has a more significant improvement than the other two groups which means host limbs enjoy a better range of motion; the relative amounts of NF200 and GAP43 in F-SHH group are much higher than the other two, while its GFAP possesses a lower relative amount than the other two, and its neuron arrangement is better than the two groups having no transplantation or having the transplantation of fibrin glue.

4 Conclusion

Compared with transplanted fibrin glue, the controlled-release SHH fibrin scaffold transplantation will recover the complete transection of spinal cord in rats better. Based on such mechanism, a further treatment for patients suffering a spinal injury can be explored in clinical practice that is to use the controlled-release SHH fibrin scaffold transplantation for the recovery of spinal cord injury in human.

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