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Absorbable collagen sponge combined with recombinant human basic fibroblast growth factor promotes nerve regeneration in rat sciatic nerve

Cheng-Can Yao · Ping Yao · Hao Wu · Zhen-Gang Zha

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Abstract Recombinant human basic fibroblast growth factor (rhbFGF) is a peptide with many bioactivities such as promoting proliferation and migration of various cells. It plays an important role in neuroprotection and enhancement of nerve regeneration. Due to its short half-life in the body, local administration by injection is limited. To prolong the bioactivity of rhbFGF and to enhance its biological effects, absorbable collagen sponge was used as matrixes and carriers for controlled release of rhbFGF. The effects of rhbFGF soaked into an absorbable collagen sponge (rhbFGF/ACS) for the repair of rat sciatic nerve injury were evaluated. The functional, electrophysiological and histological examinations demonstrate the treatment with rhbFGF/ACS can enhance rat sciatic nerve repair, and its effectiveness is better than free rhbFGF alone. It is concluded the rhbFGF/ACS is a promising biomaterial to improve the repair and regeneration of sciatic nerve injury.

C.-C. Yao
College of Pharmacy, Jinan University, Guangzhou 510632, P. R. China

C.-C. Yao
Biomaterial Key Lab of the Guangdong Provincial Department of Education, Jinan University, Guangzhou 510632, P. R. China

P. Yao
Medical Collage, Jinan University, Guangzhou 510632, P. R. China

H. Wu · Z.-G. Zha
First Clinical Medical College, Jinan University, Guangzhou 510632, P. R. China

Z.-G. Zha (✉)
Department of Orthopedics, First Affiliated Hospital of Jinan University, Guangzhou 510632, P. R. China
e-mail: zhzgg@tom.com

Introduction

Basic fibroblast growth factor (bFGF) was separated and purified from bovine pituitary in 1974 by Gospodarowicz [1], recombinant bFGF was manufactured in large-scale quantity through biotechnology including genetic engineering method and cell culture technologies in succession. This dramatically promoted its development of pharmaceutical and clinical studies [2, 3]. bFGF has many bioactivities such as stimulating proliferation and differentiation of various cells from the mesoderm, and plays key roles in embryonic development, tissue repair and wound healing, neuroprotection and never regeneration etc. [4–7].

However, the short half-life $T_{1/2}$ of bFGF and rapid deactivation at local site in the body at 37 °C result in low efficacy. So, bFGF is limited to widely apply in treatment of many diseases in many forms, mainly injection. An ideal method is controlling the release of bFGF with some materials in vivo to achieve high efficacy. Due to the special biological properties such as biocompatibility, bioabsorbency and biodegradability, collagen sponge seems to be a good candidate as delivery carrier with sustained-release matrix of bFGF [8, 9].

In this study, recombinant human bFGF (rhbFGF) was mixed with collagen solution, and this mixture was freeze-dried. The effects of the rhbFGF soaked into this absorbable collagen sponge (rhbFGF/ACS) on the regeneration of rat sciatic nerve injury were investigated.

Material and methods

Preparation of rhbFGF/ACS

The collagen was extracted from bovine tendon and the concentration was adjusted to 0.4% by weight-to-weight. An aqueous solution of rhbFGF (provided by the Biopharmaceutical R&D Center of Jinan University) in normal saline solution was mixed into the collagen solution with slow stirring. This complex solution was poured into stainless steel molds and freeze-dried to form rhbFGF/ACSs at –80 °C for 24 h. Each piece of rhbFGF/ACS contained 20 μg rhbFGF. As for controls, empty collagen sponges were prepared by the addition of normal saline solution without rhbFGF.

Animal experiment

Eighty adult Sprague-Dawley rats, weighting 200–500 g, were purchased from the Experimental Animal Center of Guangdong Province, and were randomly divided into five groups, i.e., the normal saline solution group, the ACS group, the free rhbFGF group, the rhbFGF/ACS group and the sham operation group.

Rats were prepared for surgical with anesthesia. Anesthesia was induced with an intraperitoneal injection of 2% mebumal sodium (40 mg/kg) intramuscularly. The rat skin was cleansed with 70% ethanol, and cut the lateral back at right hind limb longitudinally. The sciatic nerve was identified and cross cut off 8 mm far from the inferior border of the piriformis muscle. Then the epineurium of the impaired sciatic nerve and the skin were carefully sutured with 11–0 micro-suture monofilament. All these operative procedures were performed under a surgical microscope. The rhbFGF/ACS was placed onto the rat sciatic nerve defect. As controls, the empty collagen sponge and 10 μL of normal saline solution without rhbFGF (20 μg) were applied to the sciatic nerve defect. As controls, the empty collagen sponge with rhbFGF (20 μg) with 10 μL of normal saline solution, and (10 μL) free rhbFGF, (10 μL) for saline solution alone were applied to the sciatic nerve defect.

Assessment of sciatic nerve repair

The sciatic nerve function index (SFI) of rats was assessed [10] and the sciatic nerve conductive velocity (NCV) was determined [11] at 2, 3, 4 and 5 weeks after operation respectively. Sciatic never specimens were fixed and embedded in paraffin and sectioned at 5 μm in thickness. The sections were prepared to cut and stained with hematoxylin-eosin (HE) at 5 weeks after operation. The histological sections were observed at a magnification of 40× by a light microscope.

Statistical analysis

Experimental results were expressed as means ± standard deviation. All the data were analyzed by one-way analysis of variance (ANOVA) to assess statistical significance between experimental groups.

Results

Effects on the sciatic nerve function index

The SFI was assessed on the basis of the sham-operation group, and the normal level of SFI was considered as 0. The SFI was a negative value, and a higher SFI meant the better function of the sciatic nerve. Figure 1 shows that the SFI of injured sciatic nerve in the free rhbFGF group or in the rhbFGF/ACS group was significantly higher than the normal saline group or the ACS group at 2, 3, 4 and 5 weeks after operation respectively ($p < 0.01$). Furthermore, the SFI of injured sciatic nerve in the rhbFGF/ACS group was significantly higher than the free rhbFGF group at 2, 3, 4 and 5 weeks after operation respectively ($p < 0.01$). It indicated that the injured rats treated with rhbFGF/ACS had the better sciatic nerve function compared with the rats treated with free rhbFGF. Moreover, the sciatic nerve function could not be improved by treating with normal saline or ACS group.

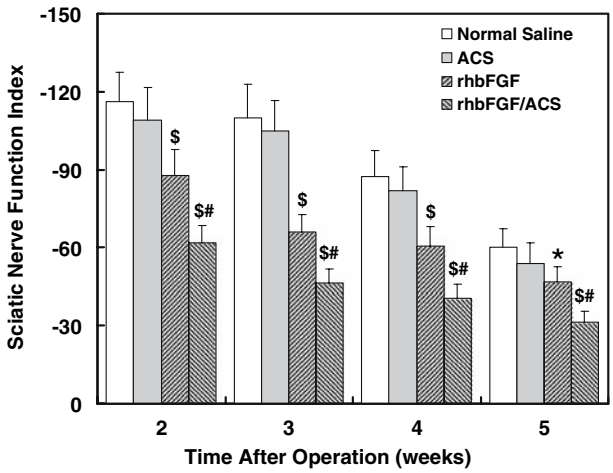

Fig. 1 Effects on the sciatic nerve function index (SFI) of injured sciatic nerves. There are significant difference ($^{*}p < 0.01$, $^{\$}p < 0.001$) of the SFIs between in the free rhbFGF or rhbFGF/ACS group and in the normal saline or ACS group at the same time; there are significant difference ($^{\#}p < 0.01$) of the SFIs between in the free rhbFGF group and in the rhbFGF/ACS group at the same time

Effects on the sciatic nerve conductive velocity

Figure 2 shows the NCV of injured sciatic nerve of rats in the sham-operation group was the fastest and the normal saline group was the lowest in all five groups at 2, 3, 4 and 5 weeks after operation. The NCV in the rhbFGF/ACS group was close to the sham-operation group. The NCV in the free rhbFGF group or in the rhbFGF/ACS group was significantly faster than the normal saline group or the empty collagen sponge group ($p < 0.01$), and the NCV in the rhbFGF/ACS group was significantly faster than the free rhbFGF group at 2, 3, 4 and 5 weeks after operation respectively ($p < 0.01$).

Histological evaluation

Figure 3 shows the histological cross section of the far-end of injured sciatic nerve at 5 weeks after operation under different conditions Several changes were observed in the normal saline group and in the ACS group, i.e., the degeneration of sciatic nerve fibers, the decrease in the density of nerve fibers and in the thickness of myeline sheath, and the reduction of the size of neuraxon and the number of Schwann's cells. Where the free rhbFGF had been applied, the thickness of myeline sheath and the density of nerve fibers decreased slightly compared with the sham-operation group. In contrast, where the rhbFGF/ACS had been applied, both the thickness of myeline sheath and the density of nerve fibers were close to those in the sham-operation group. The implanted collagen sponge had completely degraded and there was no residual collagen sponge visible at the defect. This indicated the free rhbFGF could improve the repair of sciatic nerve to some extent, whereas the effectiveness of rhbFGF/ACS for sciatic nerve wound repair was better than free rhbFGF alone.

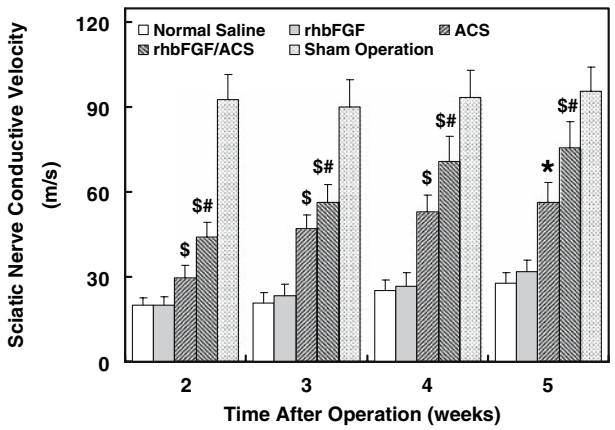

Fig. 2 Effects on the sciatic nerve conductive velocity (NCV) of injured sciatic nerves. There are significant ($^{*}p < 0.01$, $^{\$}p < 0.001$) difference of the NCVs between in the free rhbFGF or rhbFGF/ACS group and in the normal saline or ACS group at the same time; there are significant difference ($^{\#}p < 0.01$) of the NCVs between in the free rhbFGF group and in the rhbFGF/ACS group at the same time

Discussion

The bFGF can widely improve the proliferation and differentiation of cells from the mesoderm and the nerve ectoderm. It is extensively used in fields of repair of burns, wound, nerve and vessel injury [2–4]. It has demonstrated that bFGF enhanced the development of nerve system, stimulates growth of nerve cells, reduces the apoptosis of nerve cells and improves repair of nerve injury [7, 12, 13]. But the half-life period ($T_{1/2}$) of bFGF in the body is generally too short to exert its biological activity effectively when injected in the free from. It is important to study how to effectively transport bFGF to the action site, to sustain release and to enhance the efficacy over a period of time in vivo [14].

Collagen, being a major protein of connective tissues in animals, is widely used in medical application. It plays an important role in the formation of tissues and organs, and is involved in various cells in terms of their functional expression. Collagen exhibits low antigenicity, excellent biocompatibility, biodegradability and bioresorbability and becomes a superior drug carrier to control sustain release of proteins or other active products [8]. It was shown that coating of a collagen sponge with bFGF could facilitate early dermal and epidermal wound healing [15], and it was also show that acidic fibroblast growth factor (aFGF) delivered through a collagen scaffold could enhance the wound healing response of full-thickness skin defects [16]. Park et al. used collagen sponge containing antibiotics to skin wounds to prevent infection and to improve healing [17]. Moreover, Still et al. show that a collagen sponge supporting live human allogeneic skin cells could stimulate accelerated skin regeneration and wound healing of burn patients [18]. In this study, the collagen was used as a carrier to transport rhbFGF to the injury site in the body and to control the release of rhbFGF via biodegradation.

The functional, electrophysiological and histological examinations demonstrated that the treatment with rhbFGF/ACS could enhance rat sciatic nerve repair, and its effectiveness was better than that of free rhbFGF. Collagen sponge compounded with growth factors was an ideal biomaterial to improve control release of protein drugs, and would carry the future development and uses in wound repair and tissue engineering.

In summary, collagen sponge loaded with rhbFGF enhanced regeneration in rat sciatic nerve defects, and its effectiveness was better than injection of free rhbFGF alone. Slowly degradation of collagen sponges achieved the long-term release of rhbFGF in vivo, improved the

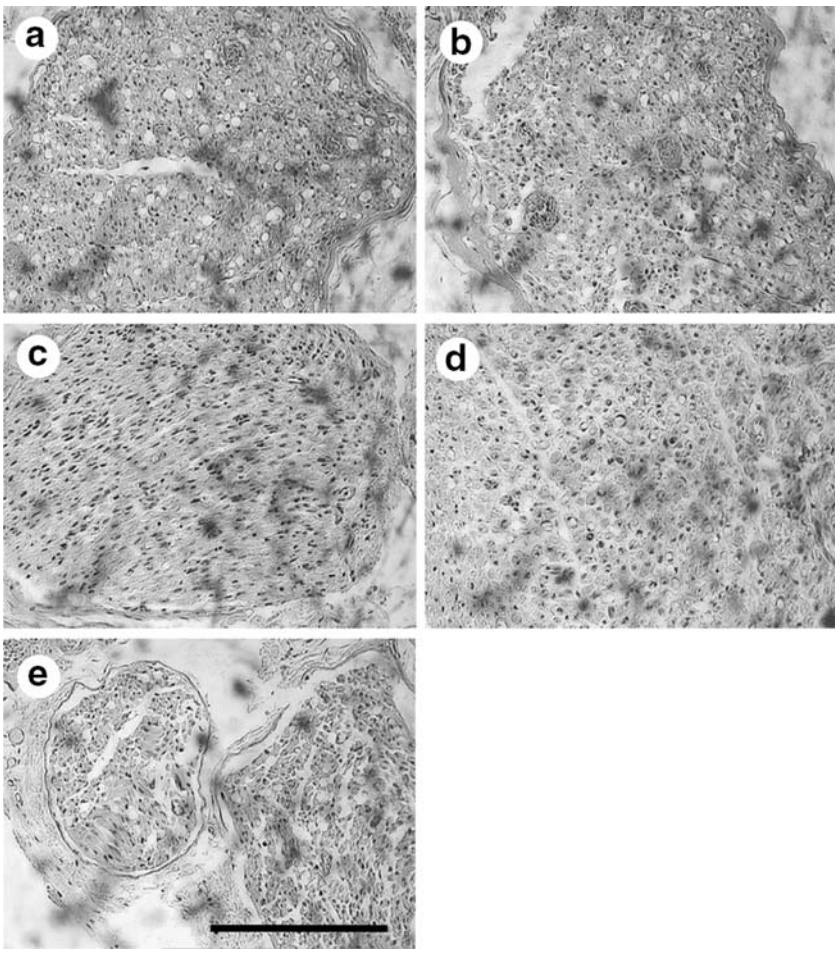

Fig. 3 Histological cross-sections of injured rat sciatic nerve 5 weeks after treatment with normal saline solution (**a**), empty collagen sponge (**b**), free rhbFGF (**c**), rhbFGF/ACS (**d**) and in sham-operation group (**e**). (bar = 50 μm)

repair of sciatic nerve for a long period, and finally enhanced the sciatic nerve regeneration.

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