Injury, Int. J. Care Injured (2008) **39**, 1414—1420

ELSEVIER

INJURY
INTERNATIONAL JOURNAL OF THE CARE OF THE INJURED

www.elsevier.com/locate/injury

Siatic nerve regeneration in rats stimulated by fibrin glue containing nerve growth factor: An experimental study

Gao Chunzheng [a], Ma Shengzhong [a,*], Ji Yinglian [b], Wang Ji-e [a], Li Jianmin [c]

[a] *Department of Orthopaedics, The Second Hospital of Shandong University, 247# Beiyuan Road, Jinan 250033, Shangdong, China*
[b] *Qianfoshan Hospital of Shangdong Province, Jinan 250012, China*
[c] *Department of Orthopaedics, Qilu Hospital of Shandong University, Jinan 250012, China*

Accepted 7 May 2008

KEYWORDS
Peripheral nerve;
Regeneration;
Fibrin glue;
Nerve growth factor

Summary

Objective: To study the effects of fibrin sealant containing nerve growth factor on the peripheral nerve regeneration.
Study design: A four-group experimental design with repeated measures on one factor was used.
Methods: Fibrin glue (FG) containing NGF was injected into the site of end-and-end sutured peripheral nerve (sciatic nerve) (group I: NGF + FG), meanwhile three control groups were set-up: group II (NGF), group III (FG), and group IV (normal saline). Observation to the function and morphology of the sciatic nerve was carried out at the end of 4, 8, 12 weeks postoperation. Data were analyzed using ANOVA, with the appropriate post hoc between-groups comparison test.
Results: Electrophysiological testing. The NAP and NCV of group I (NGF + FG) were greater than those of group II (NGF), group III (FG), or group IV (normal saline) ($p < 0.05$). *Sciatic functional index* (*SFI*). It began to ameliorate at 4 weeks post-operation and SFI increased as time went on. And the SFI in group I (NGF + FG) was better than those in group II (NGF), group III (FG), or group IV (normal saline) ($p < 0.05$). *Morphology.* In the MGF-stained sections, dissociated myelin debris was less and regenerated nerve fibres were in larger quantities in group I (NGF + FG) than in other groups. In the HE-stained sections, regenerated nerve fibres distal to anastomosis significantly increased, and axon and myelin had a clearer outline in group I (NGF + FG) than in other groups. Electron microscopy indicated that the

* Corresponding author. Tel.: +86 531 85875482.
E-mail address: mszfirst@yahoo.com.cn (M. Shengzhong).

0020-1383/$ — see front matter
doi:10.1016/j.injury.2008.05.010

regenerated nerve fibres were more mature and the development of the axons was greater in group I than in other control groups.
Conclusions: FG can be used as carrier of exogenous NGF, and they can provide synergistic effects for the peripheral nerve regeneration after the integration of the two.

Complete regeneration is commonly difficult in peripheral nerve injury, though microsurgical repair techniques have been well developed. This may be because of the lack of local neurotrophic factors and the extracellular matrix for the factors to act, which leads to the difficulty in the micro-environmental formation for peripheral nerve regeneration.[14]

Nerve growth factor (NGF), the first discovered bioactive factor in the nerve growth factors family, plays important roles in the development and regeneration of nerve system.[7] It has been found that administration of exogenous NGF to the end of nerves can protect spinal ganglion neurons after peripheral nerve injury,[11] and on the other hand, motor neurons can be significantly protected, and NGF can promote axonal extension and myelination.[16] However, exogenous NGF has a short half time in the body (2.4 min). Its activity will soon be lost and can be affected by multifactors such as temperature and pH value,[5] so many techniques, such as particulate technology,[2,13] stent technology[10] and coated cells,[9] have been adopted to achieve the maximum effect of exogenous NGF by maintaining its activity and release so that NGF plays a longer role in the process of nerve regeneration.

Studies on the stent technology showed that the extracellular matrix (ECM), mainly the basement membrane, is also an important factor affecting the peripheral nerve regeneration. Fibronectin and laminin can be the stent in nerve regeneration, and provide a good environment and guarantee for proliferation of the secreted neurotrophic factors and the selective growth of nerve fibres.[9]

The main component of synthetic fibrin glue (FG) contains mainly fibrinogen. It can be used as carrier of exogenous NGF and after the integration of the two could they provide synergistic effects for peripheral nerve regeneration? To address these questions, the authors undertook the following experimental study: exogenous NGF embedded in the FG was injected into anastomosis of rat sciatic nerve to observe the nerve regeneration.

Materials and methods

Animals and grouping

The study was agreed by the Animal Welfare Committee of Shandong University. There were 96 Wistar rats with body weight of 250—300 g. They were randomised into 4 groups with 24 animals each: group I (NGF + FG), group II (NGF), group III (FG), and group IV (normal saline).

Surgical technique

The animals were anaesthetised with 20% intraperitoneal urethane. Under aseptic conditions the left sciatic nerve was exposed. It was cut above the sciatic nerve bifurcation which was 0.5 cm under the ischial tuberosity. End-to-end suture was carried out with 10-0 noninvasive suture. In addition drugs were applied as follows: NGF—FG mixtures (4 mg/L) in group I (NGF from the U.S. Biological Engineering Ltd.; FG from Guangzhou Special Technology Ltd.); NGF (4 mg/L) in group II; FG (4 mg/L) in group III; normal saline in group IV. Injection volume was 0.5 ml. Muscle and skin were sutured. After operation, the animals were kept separately. Tests were preferred at the end of 4, 8, and 12 weeks following surgery.

Test on the function and morphology of the sciatic nerve

Electrophysiological testing

The specimens were harvested from the peroneal nerve bifurcation of the piriformis margin. A stimulating electrode was connected to the proximal and a guiding electrode was connected to the distal end. Stimulating broad was −0.2 ms, and intensity was 0.2 V. The nerve action potential (NAP) was recorded and its wave amplitude and width, and nerve conductive velocity (NCV) was calculated.

Sciatic functional index (SFI)

After the rats had touched foot blue-black ink, they passed through 8.2 cm × 42 cm wooden black box with two to three times of repeat. The clear and integral footprints were surveyed. Bain formula was used to obtain the SFI.[9] The normal value of SFI is 0, and SFI of 0 ± 11 was set as normal nerve function. SFI of −100 was complete loss nerve function.

Morphology

The anastomotic part was 10% formalin-fixed, paraffin embedded, HE stained, quick frozen embedded, MGF stained, optical microscopy observed to obtain the amount of regenerating