An experimental study of nerve bypass graft

XU Jie 徐杰* and LI Xue-shi 李学仕

Objective: To study the use of a nerve “bypass” graft as a possible alternative to neurolysis or segmental resection with interposition grafting in the treatment of neuroma-in-continuity.

Methods: A sciatic nerve crush injury model was established in the Sprague-Dawley rat by compression with a straight hemostatic forceps. Epineurial windows were created proximal and distal to the injury site. An 8-mm segment of radial nerve was harvested and coaptated to the sciatic nerve at the epineurial window sites proximal and distal to the compressed segment (bypass group). A sciatic nerve crush injury without bypass served as a control. Nerve conduction studies were performed over an 8-week period. Sciatic nerves were then harvested and studied under transmission electron microscopy. Myelinated axon counts were obtained.

Results: Nerve conduction velocity was significantly faster in the bypass group than in the control group at 8 weeks (63.57 m/s±5.83 m/s vs. 54.88 m/s±4.79m/s, P<0.01). Myelinated axon counts in distal segments were found more in the experimental sciatic nerve than in the control sciatic nerve. Significant axonal growth was noted in the bypass nerve segment itself.

Conclusion: Nerve bypass may serve to augment peripheral axonal growth while avoiding further loss of the native nerve.

Key words: Neuroma; Transplantation; Peripheral nerves

METHODS

Establishment of animal model

Twenty adult male SD rats weighing from 250 to 300 g were prepared. Anesthesia was made by abdominal cavity injection with phenobarbital sodium, 40 mg/kg. Under prone position, an 8-mm-length segment of radial nerve was harvested from the left upper limb; then cut off the skin of the posterior left lower limb and the gluteus maximus to expose the trunk of sciatic nerve. Clamp the mid-point of it with a micro-acutenaculum for 10 seconds and it could be observed under a 10× microscope in which the nerve fibers were completely mutilated and the epineurium left at the clamped site. Two windows were created with a diameter of 1.5 mm on the epineurium both proximal and distal to the clamped site, and the harvested radial nerve was coaptated with the windows in an end-to-side fashion to establish a model of nerve bypass as the experimental group. No nerve bypass graft was performed after clamping the sciatic nerve on the right side as the control group (Figure 1).

Measurement items

Being fed normally, the rats were examined as follows under anesthesia 8 weeks after the operation.
**Nerve conduction studies:** Sciatic nerves of both sides of the rats were exposed. An Italian made Esaota Biomedica Phasis electromyogram machine was used to give super electrical stimulation at the both sites of 0.8 cm proximal and distal to the neuroma on the sciatic nerves. The latent period and wave amplitude of compound muscle action potential (CMAP) are recorded on the gastrocnemius muscle of both sides and nerve conduction velocity (NCV) was calculated. All the examination process was carried out under the temperature of 28°C and 0.9% saline solution was often used to retain muscular moist state.

**The number and sectional area of medullated nerve fibers:** After the electro-neurogram examinations, nerve samples were drawn from the both sites of 0.8 cm proximal and distal to the neuroma, fixed by 10% neutral formalin, cut into 5 µm-thick cross sections and stained by hematoxylin-eosin. A Leica biological microscope was used to count the total number of medullated nerve fibers under 400-fold magnification. A TJTY-300 image analyzer was used to measure the sectional area of medullated nerve fibers under the condition of 40-fold magnified eyepiece and 0.38 µm/pixel calibration. A straight line was set through the center of the medullated nerve fiber and the sectional area on the line was measured. The mean value of the sectional area was adopted as the sectional area of the plane.

**Electron microscope observation:** Samples were taken from the both sites of 0.8 cm proximal and distal to the neuroma from the sciatic nerve and the midpoint of the bypass nerve, 2.5% glutaraldehyde fixation under 4°C, 1% osmic acid fixation, alcohol gradient dehydration, epoxy resin 618 embedding, ultrathin section, uranyl acetate and lead citrate double staining. A JEM-1200EX was used for transmission electron microscope (TEM) observation. Every sample was photographed from 3 different random visual fields under the condition of 1000 times magnification. A TJTY-300 image analyzer was used to measure the inside diameter (ai, bi) outside diameter (ao, bo), inside perimeter (Pi) outside perimeter (Po), inside area (Ai) outside area (Ao) of the elliptical myelin sheath under the condition of 40 times magnification eyepiece and 0.38 µm/pixel calibration. The thickness of the myelin sheath (mt) and the diameter ratio (d/D) of the myelinated nerve fibers were calculated according to the formula listed below (Figure 2).

\[
d = \sqrt{\frac{P_i^2 + 4n(A_i)}{P_o^2 + 6n(A_o)}}
\]

\[
mt = \frac{2(A_o - A_i)}{P_o + P_i}
\]

**Muscle wet weight and the sectional area of muscle fibers:** The gastrocnemius of both sides of the rats were harvested completely and the superficial connective tissue was removed and their wet weight was weighed on a ten thousand precision TG328A scale immediately. After that, the weighed gastrocnemius was under fixation for 48 hours, dehydration, paraffin embedding, 5 µm sectioning through the maximum sectional area, HE staining. The sectional areas of the muscle fibers were measured with a TJTY-300 image analyzer under the condition of 40-fold magnification eyepiece and 0.70 µm/pixel calibration.

**Statistical analysis**
The experimental data were expressed as x±s. Paired Student’s t test was used to compare the parameters between the left side and right side.

**RESULTS**

**Comparison of nerve conduction studies**
Eight weeks after the operation, the CMAP wave amplitude and NCV of the experimental side were superior to those of the control side, which were statistically significant (Table 1).

**The number of myelinated nerve fibers**
At the site of 0.8 cm proximal to the neuroma, there was no statistical difference on the number and sectional area of myelinated nerve fibers between the two
sides. While at the site of 0.8 cm distal to the neuroma, there was statistical difference of the number and sectional area of myelinated nerve fibers between the two sides, and the results of the experimental side were superior to the control side (Table 1, Figure 3).

**Muscle wet weight and sectional area of muscle fibers**

Eight weeks after the operation, the wet weight and sectional area of muscle fibers of the gastrocnemius of experimental side were superior to control side, which were statistically different (Table 1).

**Electron microscope inspection**

Plenty of densely arranged myelinated nerve fibers with thick myelin sheath and bulky diameter were observed under electron microscope. Plenty of regenerated myelinated nerve fibers of various sizes were observed in bypass nerve. At the site of 0.8 cm distal to the neuroma, the regenerated myelinated nerve fibers of experimental side were denser compared with control side. There was no significant statistical difference of myelin sheath thickness and d/D ratio between the two sides (Table 1).

**DISCUSSION**

**Mechanism of nerve regeneration after end-to-side anastomosis**

The history of peripheral nerve end-to-side anastomosis could date back to the very beginning of last century. But the way of nerve anastomosis was not thought highly of for its good clinical application perspective until Viterbo and his colleagues carried out a series of experimental and clinical researches on end-to-side anastomosis in 1992. The way of peripheral nerve end-to-side anastomosis is to anastomose the end of the injured nerve to the side of a kinematic nerve, or connect the injured nerve with a kinematic nerve through a section of grafted nerve. Viterbo, Lundborg and their colleagues proved that the regenerated nerve fibers of end-to-side anastomosis were derived from the lateral bud emerging from the trunk of the dynamic nerve through electrophysiology and histological study. The character, creating windows or not, anastomosing angle of dynamic nerve and the predenaturation of grafted nerve would impose some influence to lateral bud regeneration.

**Clinical significance of this research**

The reason for neuroma formation is that after the
break of nerve fibers, endoneurium, perineurium and epineurium, scar hyperplasia will happen at the site of injury; the growth of nerve fibers of the proximal end to the distal end is retarded; a tuberiform appearance is formed at last. Sunderland I degree injury is nerve apraxia without the formation of neuroma. In Sunderland II degree injury, there will be no neuroma formation for the integrity of endoneurium tube despite the break of nerve fibers. Both the I and the II degree injury could gain totally functional recovery. When the III degree injury happens, the endoneurial tube will be broken and malpositioned, and the growth of nerve fibers will be blocked, but because of the integrity of perineurium, the growth of neuroma will be confined in perineurium and will not form dilated nerve trunk, and partially functional recovery will be ensued. When the IV degree injury happens, there will be many broken and malpositioned nerve tracts. The hyperplastic scar and the fibers under growth retardation will lead to the formation of tuberiform nerve trunk. Badly functional recovery will be ensued. The neuroma formed after the IV degree injury usually appears in the advanced stage of periphery nerve compression (eg, cubital tunnel syndrome, posterior interosseous nerve compression, etc) and obstetric brachial plexus injury and so on.6

At present, the ways to cure them are to decompress the compressed nerve or perform nerve grafting after neuroma resection,6,7,8 but both of the two methods will cause further loss of original nerve function. This study proved that there were regenerated nerve fibers of different sizes in the bypass nerve through electron microscope. It is testified through nerve conduction study and histological study that after the bypass nerve grafting, the NCV of neuroma is faster, and the amount of regenerated nerve fibers, the section area of dominated muscle fibers and the wave amplitude of CMAP are increased. Compared with the traditional nerve grafting, nerve bypass grafting has several advantages: 1) maintenance of the original nerve function, 2) without the secondary injury to the dynamic nerve, 3) obtaining more amount of regenerated nerve fibers through many bypass grafting on many nerve trunks. Therefore, nerve bypass grafting could be a clinical operation option to repair partial injury of periphery nerve. The author’s opinion is that if the neuroma is formed just on one side of the nerve trunk, there are continuous nerve tracts on the other side and most functions of the dominated area exist. Neurolysis could be performed at the site of injury, but if only very limited functions exist, the neuroma of injured side should be resected and direct nerve anastomosis or nerve grafting should be performed. For the IV degree injury, especially with the formation of neuroma on the nerve trunk caused by traction, comprehensive judgement should be made according to the remained nerve function, impaired level, the degree of myatrophic, neural regeneration potential and the electrophysiological appearance during operation. When there are high-level injury and little remained nerve function, the prognosis of traditional nerve grafting after neuroma resection will not surpass that of neurolysis. Nerve bypass grafting or low-level end-to-side anastomosis will all be good alternatives.

REFERENCES


(Received October 8, 2007)
Edited by SONG Shuang-ming