Single Versus Double End-to-Side Nerve Grafts in Rats

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Purpose Although the end-to-side nerve repair technique has been used clinically, it has not yet produced consistent motor and sensory recovery in patients. The aim of this study was to investigate whether end-to-side double nerve grafts display more axonal regeneration compared with a single nerve graft in a rat lower limb preparation.

Methods The lower limbs of 96 Wister rats were used in experiments comparing single and double end-to-side nerve grafts. Left peroneal nerves were harvested and grafted between the right peroneal and tibial nerves. A single graft was attached end-to-side to the peroneal and tibial nerves through an epineural window (single graft group, n = 24). Two grafts were performed in the same manner in the double graft group (n = 24). The peroneal nerve was exposed in positive controls (n = 24) and no graft was performed in negative controls (n = 24). We recorded action potentials and moist weights of the left tibialis anterior muscle at each time point. Fluoro-Gold-labeled (Fluorochrome, Denver, CO) dorsal root ganglion neurons from L1 to L6 were counted using fluorescence microscopy and compared among the 4 groups.

Results In both single and double groups, the amplitude and the tibialis anterior muscle weight increased significantly compared with negative controls but remained lower than those measured in positive controls. There was no significant difference between single and double groups. In Fluoro-Gold-labeled neurons, there was also no significant difference between single and double groups.

Conclusions The study showed that regeneration of motor and sensory nerve fibers was possible using 2 end-to-side nerve grafts. However, there was no significant difference between single and double grafts. This might suggest a therapeutic limitation of nerve transplants using 2 end-to-side nerve grafts.

Clinical relevance Double end-to-side repair attracts both motor and sensory axons, and this results in a medium degree of recovery of function; however, double end-to-side nerve grafting does not appear to offer any advantage over a single end-to-side graft. *(J Hand Surg 2012;37A:261–269. Copyright © 2012 by the American Society for Surgery of the Hand. All rights reserved.)*

Key words End-to-side, graft, nerve repair, rat, reinnervation.

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End-to-side neurorrhaphy was described more than 100 years ago.[1] The concept had already been proposed in a book published by Letievant in 1873 as a technique for large peripheral nerve defects; however, clinical cases were not reported. Subsequent literature reported clinical cases.[2–6] However, due to the unpredictable results, the study of end-to-side neurorrhaphy was almost abandoned for more than 50 years until Viterbo reintroduced this method.[7,8] Many reports of animal models and clinical experiments of end-to-side nerve repair have since been published.[9–15]

Viterbo et al published a report of 2 end-to-side neurorrhaphies.[16] They indicated that axons grew through 2 end-to-side nerve junctions on each end of the nerve graft. They noted 4 positive muscle responses on the side of nerve repair, but that was only 44% of the 9 tested. We became interested in this technique because it is simple and could be applied clinically if it would encourage efficient nerve regeneration. We hypothesized that end-to-side double nerve grafts could regenerate more axons when compared with a single nerve graft in a rat.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 210–230 g were used (8 weeks old at the start of experiments; Japan SLC, Shizuoka, Japan). All animals were housed in the animal unit at 21°C on a 12-hour light–dark cycle (08:30–20:30) and allowed a pellet diet and tap water *ad libitum*. Experiments were performed with permission from the ethics committee of the Graduate School of Medicine, Chiba University, following the National Institutes of Health guidelines (1996 revision) for the care and use of laboratory animals.

Surgery

All procedures were performed under 2.5% halothane anesthesia in 50% oxygen and treated aseptically throughout the experiments. Under a surgical microscope, the peroneal nerve was divided on the right side of all animals. In the graft groups, the left side peroneal nerve was harvested for grafting.

In the single graft group, the proximal end of the sectioned, right peroneal nerve was inserted into the femoral adductor muscle parallel to and 1 cm away from the tibial nerve. This step minimized any axonal regrowth into the distal end of the peroneal nerve from the proximal end. After opening a 1-mm perineural window in the intact tibial nerve and the side of the peroneal nerve using a 25-gauge needle, a graft was interposed and secured using 2 epineural 10-0 nylon sutures.

In the double graft group, 2 grafts were constructed in the same manner. A group with no graft was used as a negative control. A group in which the peroneal nerve was only exposed was used as a positive control (Fig. 1). All animals were given antibiotics (500 L; Bacitracin, Chuugai Pharmaceuticals, Tokyo, Japan) by subcutaneous administration after the surgery. The wounds were closed, and the animals were allowed to recover, which was uneventful in all cases.

Experiment 1: confirmation of nerve regeneration in graft

Sixty-four male Wistar rats were used in this experiment (each group, n = 16). At various time points (4, 8, 12, and 16 wk) after the surgery, the animals were anesthetized with sodium pentobarbital (40 mg/kg, intraperitoneally) and the right sciatic nerve was divided. Biotinylated dextran amine (BDA; molecular weight, 10,000; 10% in 2.0 ìL 0.01 M phosphate-buffered saline; Molecular Probes, Eugene OR), which acts as an anterograde neurotracer, was injected into the proximal level of the tibial nerves using a Hamilton syringe attached to a glass micropipette. At 14 days after BDA injection, rats were anesthetized with sodium pentobarbital and transcardially perfused with 0.9% saline, followed by 500 mL 4% paraformaldehyde in phosphate buffer (0.1 M; pH, 7.4). The graft and the distal level of the peroneal nerve were resected, and the specimens were immersed in the same fixative solution overnight at 4°C. After being stored in 0.01 M phosphate-buffered saline containing 20% sucrose for 20 hours at 4°C, each nerve was sectioned at 16 μm thickness in the sagittal plane on a cryostat and mounted on poly-1-lysine-coated slides. Sections were incubated with Alexa Fluor 594-conjugated streptavidin (1:800; Molecular Probes, Eugene, OR). The BDA-labeled nerve axons (regenerated axons) at the grafts and the distal level of the peroneal nerve were confirmed using fluorescence microscopy (Olympus, Tokyo, Japan). One slide on 1 animal was stained. To evaluate the numbers of immunoreactive fibers, 10 random fields were selected from 1 slide, average counts for each group were calculated using total count for each animal, and mean comparisons were performed among the 4 groups.

Experiment 2: electrophysiological tests

Ninety-six male Wistar rats were used in this experiment (each group, n = 24). At various time points (5,