Experimental Study on Facial Nerve Regeneration With or Without Geniculate Ganglionectomy

Zhengmin Wang, MD; Chunfu Dai, MD, PhD; Yuhai Zhang, MD

Objective: To investigate regeneration of the distal facial nerve following nerve grafting within the tympanic segment with geniculate ganglion preservation or dissection.

Design: Randomized controlled trial.

Subjects: Twenty-three adult New Zealand albino rabbits were used in this study.

Interventions: A 2-mm tympanic segment of the facial nerve was removed, and the greater auricular nerve was harvested for grafting in all animals. In group 1 (10 rabbits), the geniculate ganglion was preserved. In group 2 (13 rabbits), the geniculate ganglion was dissected. Mastoidal and extratemporal segments of the facial nerve were harvested 3 months postoperatively for histological examination by electron microscopy.

Results: The number of myelinated axons in normal facial nerves was 1819.6±535.6. In group 1, the number of myelinated axons was 123.6±31.1, and, compared with normal facial nerves, the diameter of the regenerative axons was decreased and the sheath thickness in the regenerative fiber was diminished. In group 2, the number of myelinated axons was 515.1±103.1, while the myelin sheath thickness was proportionate to axon diameter. (Data are given as mean±SD.)

Conclusion: Geniculate ganglionectomy may improve motor axon regeneration.


Several attempts have been made at morphological and quantitative analysis of the geniculate ganglion. Previous studies indicated that the total number of ganglion cells in a single temporal bone ranged from 589 to 4183 (mean, 2162 cells). In 88% of patients, most of these cells were found in the geniculate ganglion. However, in 8% of patients, most of these cells were in the internal acoustic meatus; in 4%, the meatus and geniculate ganglion contained an equal number of cells. There was no correlation between total ganglion cell number and age or sex of the patient. The ganglion cell bodies were aggregated at the apex of the genu, close to the origin of the greater superficial petrosal nerve. These findings suggested a possible therapeutic benefit from geniculate ganglionectomy in patients with facial paralysis.

Relative to other cranial nerves, the facial nerve is particularly prone to injury because of the long distance it traverses intratemporally and extratemporally. Studies have been conducted that focus on axon regeneration and functional recovery following facial nerve injury. Nerve regeneration involves a complex interaction of neurons, Schwann cells, elements of the extracellular matrix, and a host of neurotrophic substances. With respect to surgical repair, suturing the severed nerve ends and nerve grafting have remained the procedures of choice. The immediate neural environment plays an important role. The success of neural regeneration depends on the cellular matrix components (lamina, type IV collagen, neural adhesive molecular, and others) and on neurotrophic factors, such as nerve growth factor, that are produced by the denervated target nerve. A recent study showed that electromagnetic stimulation enhances early regeneration and functional recovery. Most investigations have focused on motor fiber regeneration and facial movement.

It is poorly understood how the secretomotor fibers and gustatory fibers regenerate after facial nerve injury and whether their regeneration affects motor fiber regeneration. The purpose of the pres-
MATERIALS AND METHODS

ANIMALS

Twenty-three adult New Zealand albino rabbits of both sexes weighing 2.5 to 3.2 kg were randomly assigned to 2 groups. All animals underwent removal of a 2-mm tympanic segment of the facial nerve, and the greater auricular nerve was harvested for grafting. In group 1 (10 rabbits), the geniculate ganglion was preserved. In group 2 (13 rabbits), the geniculate ganglion was dissected. Mastoidal and extratemporal segments of the facial nerve were harvested 3 months postoperatively for histological examination.

SURGICAL PROCEDURE

All operations were performed on the left side with an operating microscope (Carl Zeiss, Inc, Oberkochen, Germany). The right side served as a control. All surgical procedures were performed under aseptic conditions. The animals were anesthetized with inhalation through intubation with 0.5% enflurane with equal parts of nitrous oxide and oxygen. The otic vesicle was exposed postauricularly. Through the surgical fenestration, the incus was removed and the tympanic fallopian canal was identified above the footplate of the stapes. The fallopian canal was opened with a diamond burr, and 2.0 mm of the facial nerve was removed. The nerve graft was carefully inserted in the opened canal between the cut ends of the facial nerve. The donor nerve for grafting was the greater auricular nerve.

In addition to the above procedures, the animals in group 2 had the malleus head removed to expose the geniculate ganglion. The geniculate ganglion and the greater superficial petrosal nerve were excised, and the main trunk of the facial nerve was preserved. To prevent regeneration of the intermediate nerve to the distal segment of the facial nerve, bone wax was replaced in the geniculate ganglion. Antibiotics were administered to minimize the possibility of infectious complications. Three months postoperatively, all animals were placed under deep general anesthesia, the otic vesicle was opened, the parotid gland was retracted forward slightly, the stylomastoid foramen was dissected, and the mastoidal and extratemporal segments of the facial nerve were harvested for electron microscopic evaluation. The methods and protocol of the study were reviewed and approved by the Institutional Animal Care and Use Committee of Shanghai Medical University, People’s Republic of China.

HISTOLOGICAL EXAMINATION

For electron microscopic evaluation, tissues were immersed in 3% glutaraldehyde for 2 hours and then in 1% osmium tetroxide for 1 hour. Dehydration in a series of graded ethanol was followed by gradual infiltration with epoxy (Embed-812; Electron Microscope Sciences, Tokyo, Japan). Semithin sections were made and stained with toluidine blue O for light microscopic examination, and ultrathin sections were stained with uranyl acetate and lead citrate for electron microscopy (JEM-2000CX; JEOL Co, Tokyo, Japan). Axon count and size and distribution of unmyelinated and myelinated fibers were determined by electron microscopic examination.

Analyses to determine the statistical significance of the difference in the axon count of the facial nerve between group 1 and group 2 were performed using t tests with commercially available software (STAT VIEW, version 5.0; Abacus Concepts Inc, Berkeley, Calif).

RESULTS

In normal facial nerves, myelinated axons were evenly distributed. Sheath thickness was proportionate to axon diameter (Figure 1), and the number of axons was 1819.6 ± 535.6.

In group 1, regenerative myelinated fibers and unmyelinated fibers were identified in the mastoidal segments. However, the number of myelinated axons was 123.6 ± 31.1, which was much fewer than were found in normal facial nerves. In addition, the diameter of regenerative axons was decreased, and the sheath thickness in regenerative fibers was diminished (Figure 2). Extratemporal segments were almost completely composed of connective tissue, with fewer myelinated axons than were found in the mastoidal segments. Myelinated axons were diffusely distributed throughout the extratemporal segments. It is estimated that only one eighteenth to one twelfth of myelinated axons are used to innervate muscle.

In group 2, a large number of regenerative myelinated axons was found in the mastoidal segments. The myelin sheath thickness was proportionate to the diameter of the axons (Figure 3). No unmyelinated axons were seen. In the extratemporal segments, myelinated axons were evenly distributed. The number of myelinated axons was 515.1 ± 103.1, which is about one quarter to one third of that in normal facial nerve. Proliferation of connective tissue was noted among axons. However, comparing group 1 with group 2, the number of regenerative myelinated axons was increased significantly (P < .001) in group 2.

Data are given as mean ± SD.

COMMENT

It is well-known that there are somatic motor, gustatory, and secretomotor fibers within the facial nerve. Early regenerative gustatory fibers in chorda tympani and secretomotor fibers are unmyelinated. Mature regenerative gustatory fibers and somatic motor fibers are myelinated. An anatomical study has indicated that the total number of myelinated nerve fibers in the facial nerve varies from 7500 to 9370, depending on the anatomical level of the nerve segment. The greatest number of nerve axons was found at the level of the middle of the mastoidal portion. The peak diameter of the facial nerve axon was between 4 and 6 µm. The number of facial nerve fibers...
decreased with the age of the patient. Further investigation showed that a significant proportion (15%-20%) of the fiber composition in the facial nerve trunk and its peripheral branch is nonmotor. Bruesch, using chromatolytic technique in cats to trace the distribution of the afferent fibers through the facial nerve branches, found that 20% of fibers traversed the greater superficial petrosal nerve. The remainder was distributed in the chorda tympani (45%), posterior auricular rami (21%), branches to mimetic muscle (8%), deep cervical branch (5%), and nerve to the stapedius (1%). Somatic motoneurons are in the facial motor nucleus, which is located in the brainstem. Secretomotor neurons are situated in the superior salivatory nucleus. Sensory neurons of the gustatory fibers localize in geniculate ganglia via the chorda tympani nerve, supplying taste buds in the tongue.

In this study, we demonstrated axonal regeneration following facial nerve grafting with or without geniculate ganglionectomy. With geniculate ganglion preservation, an increase in regenerative gustatory fibers was identified in mastoidal segments following facial nerve transection. There were more unmyelinated fibers than myelinated fibers. In contrast, with geniculate ganglionectomy, a large number of myelinated axons was found in the mastoidal segments, the number of regenerative axons was similar to that of the control side, and there were no gustatory fibers. It is speculated that geniculate ganglionectomy has a positive effect on motor axon regeneration. The results may be explained by the fact that gustatory fiber is a C-type fiber and grows quickly following injury, and that gustatory neurons are in geniculate ganglia. However, compared with gustatory fibers, motor fibers must traverse a longer distance from the facial motor nucleus to the injury site. Therefore, it is advantageous for the regenerative gustatory fibers in the band of Bünge to regenerate. After geniculate ganglionectomy, the band of Bünge gustatory fibers served as guides for motor fiber regeneration.

Ylikoshi et al observed degenerative changes in the distal stump of the human facial nerve. Their results showed that the sensory component of the facial nerve had a normal appearance when the facial nerve was severed at the internal acoustic meatus with the geniculate ganglion left intact. When the geniculate ganglion was damaged, the normal-looking sensory component of the facial nerve was absent from the distal stump. When the entire tympanic portion of the facial nerve was interrupted, most of the endoneural tube had thin unmyelinated fibers in the distal stump around the foramen, and few myelinated fibers appeared intact. It may be that regenerative sensory fibers interfere with the regeneration of the motor fibers. This observation was consistent with the findings of our study.

Moreover, in group 2, no unmyelinated fibers were found in the mastoidal segments following geniculate ganglionectomy. In addition, compared with group 1, the number of regenerative myelinated fibers was significantly increased in the mastoidal segments. This suggests that, without the effect of nonmotor fiber, more motor fiber may be regenerated.

From our experimental data, it seems reasonable to conclude that geniculate ganglionectomy is associated with improvement of motor axon regeneration. Earlier studies proposed geniculate ganglionectomy as a means of excising the cell bodies of aural cutaneous pain afferents in patients with geniculate neuralgia. These re-

Figure 1. Electron microscopic examination of a mastoidal segment of normal facial nerve shows myelinated axons that are evenly distributed and whose sheath thickness is proportionate to the axon diameter (original magnification ×960).

Figure 2. In a mastoidal segment of facial nerve in group 1, myelinated axons (arrowhead) and a greater number of unmyelinated axons (star) were noted under electron microscopy (original magnification ×460).

Figure 3. In group 2, electron microscopy shows a large number of myelinated axons in extratemporal segments of facial nerve following geniculate ganglionectomy. There were fewer myelinated axons than were found on the control side. Fibers were evenly distributed, with a proliferation of connective tissue (arrowhead) among the axons (original magnification ×12000).
ports showed that this surgery benefited these patients. A histological basis for the use of geniculate ganglionecomy as a treatment for geniculate neuralgia was thereby established. It is recommended that geniculate ganglionectomy be performed when facial nerve decompression or grafting is needed in patients with facial paralysis, as it may enhance motor axon regeneration and improve the recovery from facial palsy.

The present study only investigated motor axon regeneration following geniculate ganglionectomy. Further studies should be undertaken to focus on facial movement evaluation by electrophysiologic testing.

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Corresponding author and reprints: Chunfu Dai, MD, PhD, Department of Otolaryngology, Eye, Ear, Nose, and Throat Hospital, Shanghai Medical University, Shanghai 200031, People's Republic of China (e-mail: daichf@online.sh.cn).

REFERENCES


