Autogenous standard versus inside-out vein graft to repair facial nerve in rabbits

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**Objective:** To evaluate autogenous vein grafts and inside-out vein grafts as conduits for the defects repair in the rabbit facial nerves.

**Methods:** The 10 mm segments of buccal division of facial nerve were transected for 48 rabbits in this study. Then the gaps were immediately repaired by autogenous vein grafts or inside-out vein grafts in different groups. All the animals underwent the whisker movement test and electrophysiologic test during the following 16 weeks at different time points postoperatively. Subsequently, the histological examination was performed to observe the facial nerve regeneration morphologically.

**Results:** At 8 weeks after operation, the facial nerve regeneration has significant difference between the experimental group and the control group in electrophysiologic test and histological observation. However, at the end of this study, 16 weeks after operation, there was no significant difference between inside-out vein grafts and standard vein grafts in enhancing peripheral nerve regeneration.

**Conclusion:** This study suggest that both kinds of vein grafts play positive roles in facial nerve regeneration after being repaired immediately, but the autogenous inside-out vein grafts might accelerate and facilitate axonal regeneration as compared with control.

**Key words:** Facial nerve; Nerve regeneration; Tissue graft; Tubulization

**METHODS**

Animal model and experimental grouping

In the experiment, 48 male New Zealand white rabbits, weighing 2.0-2.5 kg, were used. These rabbits were randomly divided into three groups as follows: Group A with standard vein graft (SVG, n=21), Group B with inside-out vein graft (IOVG, n=21) and Group C as normal control (NC, n=6).

In Group A, all the left buccal division of facial nerves were transected to make 1 cm nerve tissue defect separately and repaired immediately using autogenous facial vein grafts. In Group B, the autogenous facial vein grafts were turned inside out before they were applied to bridge the facial nerve gaps. The rabbits in Group C just served as normal control.
All experimental animals were anesthetized by intravenous injection of 3% pentobarbital sodium for 1.2-1.5 mg/kg. Furthermore, the animal’s face was shaved and prepared with Betadine. Under the sterile condition, the left facial nerve was exposed by a superior mandibular incision for 2.5 cm long. Depending on the laxness of facial skin, the left facial vein graft (at the length of 14 mm) can be harvested at the same time without another extra incision. Subsequently, the inside-out vein graft was made following the protocol described by Wang. On the other hand, once the left buccal branch of facial nerves had been freed completely from the surrounding connective tissue, it was transected to make a 1 cm defect. All vein grafts, whether be invaginated or not, were interposed between the nerve stumps, which were inserted into the vein lumen with 2 mm long. This repair step was accomplished by taking the vein wall and the epineurium sewed up together with 9-0 nylon sutures. The skin incisions were closed by 3-0 nylon sutures.

Whisker movement
The functional recovery assessment was performed in different groups respectively according to the following scale: 0, complete paralysis with whiskers flattened backward; 1, slight whisker motion; 2, mild motion; 3, moderate motion of whiskers and lips; 4, full motion of upper lips and whiskers. For single blind evaluation, three technical assistants who did not know the repair allocation evaluated the left facial nerve function weekly since four weeks after operation. The mean values of Groups A and B at different time points were processed and compared by the statistic analysis.

Electrophysiological testing
The electrophysiological test was performed at 4, 8, 16 weeks postoperatively randomly in every seven rabbits of Groups A and B. Under the general anesthesia, rabbits facial nerves (buccal division) on the left sides were exposed and dissected free from fibrous tissue and branches once again. A heating lamp was used to maintain the body temperature for rabbits at approximate 37°C. The electrophysiological test was performed by an electromyography system (MEB-2200 Nihon Konhden, Japan). The stimulating electrode was placed under the distal nerve stump and recording one was pricked into the ear. Consequently, data which reflects left facial nerve function, including peak latencies (ms), amplitudes (mv) and conduction velocity (m/s) in each group were measured and compared.

Histological and morphological analysis
After the electrophysiological examination, the selected experimental animals were sacrificed under overdose anesthesia at the above time points. The grafted vein segments were carefully dissected and removed. The middle and distal parts were fixed in 0.1 mol phosphate-buffered saline (PBS, pH=7.4) containing 1% glutaraldehyde for 2 hours. After post-fixation with 2% osmium tetroxide for another 12 hours at 4°C, the nerve specimens were dehydrated and embedded in Epon 812 resin. Semi-thin sections were cut off and stained by toluidine blue, and finally cover slipped. These slides were examined with a light microscope (DMI6000B, Leica, Germany) in twenty different fields and analyzed with an image-analyzing software (Image Pro Plus, Version 4.5, Media Cybernetics, USA) for quantitative axon counts and measurements of myelin sheath. For electron microscopy, ultrathin sections (at the thickness of 70-90 nm) in mesh grids were stained with uranyl acetate and lead acetate and examined with a transmission electron microscope (H-600IV, Hitachi, Japan).

Statistical analysis
All data in three groups at every time point were processed and compared statistically by the Student’s t test using statistical software package SPSS 13.0. Analysis of variance (ANOVA) was also performed. P<0.05 was considered significantly different.

RESULTS

Function assessment
All rabbits tolerated the operations well and the surgical wounds healed primarily. The animals remained in a completely paralytic state without any whisker movements in the left sides after operation. Four weeks postoperatively, obvious paralysis of ipsilateral upper lip was visible. Most rabbits had regained some whisker movements, with function having recovered since 5 weeks postoperatively. Eight weeks after surgery, mild movement of left upper lip could be observed. Treatment with the inside-out vein grafts resulted in significant recovery of function from 8 to 12 weeks as compared with control group. Although lip movements in the left side have improved remarkably at 16 weeks, they were still not as strong as the right normal side in
Groups A and B. Furthermore, the data showed that the two groups had no significant difference at the end of study. Fig. 1 demonstrates the time course of whisker movement recovery after nerve operation. This test indicated that IOVG had more positive effect at early stage of facial nerve regeneration.

Electrophysiological evaluation

The axonal loss induces a significant decline in nerve amplitude, and demyelination leads to an increase in latency as well. The facial nerve transaction produced a combination of delayed latency and diminished amplitude. The results of electrophysiological study (Table 1) demonstrated that the two experimental groups had significant difference in the early regeneration stage, particularly in the 4 weeks and 8 weeks after repair surgery. Although the facial nerve function recovered well at 16 weeks and there was no significant difference between the groups. Data about the latency, amplitude and conduction velocity were all below the normal nerve.

Histological and transmission electron microscopy observations

Histological observations showed that there was no significant difference at 4 weeks after surgery between Groups A and B. Both groups displayed that the regenerated axons appeared in the center of different vein grafts. They were slight, irregularly distributed and surrounded by the connective tissue. However, at 8 weeks postoperatively, the number of myelinated nerve fibers increased obviously just in Group B, not in Group A. Furthermore, the diameter of myelinated fibers and myelin thickness were relatively smaller in Group A ($P<0.05$). At 16 weeks after operation, there were a greater number of myelinated and well-orientated axons with larger diameter and thicker myelin sheath in Groups A and B. Most of nerves appeared to have relatively normal morphological structures, but the proportion of small axons was somewhat higher than control group ($P<0.05$). No remarkable difference in histological findings was noted between these two groups ($P>0.05$). Observations obtained from the electron micrograph demonstrated that there were many hypertrophic Schwann cells in the regeneration period. With the new axon maturated, the number of Schwann cells was sharply decreased. At the later stage, there were no differences on myelin thickness and axon amounts between Groups A and B from the light and electron microscopy analysis (Figs. 2-3).

<table>
<thead>
<tr>
<th>Items</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>16 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency (ms)</td>
<td>2.04±0.11</td>
<td>2.54±0.06</td>
<td>1.72±0.07</td>
</tr>
<tr>
<td>t=18.29, $P&lt;0.001$</td>
<td>t=10.677, $P&lt;0.001$</td>
<td>t=1.069, $P&gt;0.05$</td>
<td></td>
</tr>
<tr>
<td>Amplitude (mv)</td>
<td>2.13±0.00</td>
<td>1.54±0.14</td>
<td>4.95±0.17</td>
</tr>
<tr>
<td>t=5.258, $P&lt;0.001$</td>
<td>t=2.715, $P&lt;0.005$</td>
<td>t=1.591, $P&gt;0.05$</td>
<td></td>
</tr>
<tr>
<td>Conduction Velocity (m/s)</td>
<td>9.09±0.06</td>
<td>5.61±0.21</td>
<td>18.86±0.63</td>
</tr>
<tr>
<td>t=73.18, $P&lt;0.001$</td>
<td>t=34.231, $P&lt;0.001$</td>
<td>t=7.086, $P&lt;0.05$</td>
<td></td>
</tr>
</tbody>
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*P<0.05, IOVG group is compared with SVG group.
Fig. 2. Light micrographs of semi-thin sections stained with toluidine blue to the distal cables of the two experimental groups (SVG: A, C; IOVG: B, D) at 8 (A, B) and 16 weeks (C, D) postoperatively (×630). The upper pictures showed that there were sparse and unevenly myelinated nerve fibers in early stage of regeneration and the significant difference existed between two groups. The lower pictures showed that more myelinated fibers with larger diameter and myelin thickness in the later period and no remarkable difference existed in two groups.

Fig. 3. Transmission electron micrographs in the distal stumps of SVG (A, B) and IOVG (C, D) at different time points (×8000). The upper pictures demonstrated that more myelinated new axons were observed in IOVG (B) than SVG (A) at 8 weeks. Furthermore, A was significantly mature than B. The lower pictures showed that the diameter and myelin thickness of the new nerve fibers increased, but the number of hypertrophic Schwann cells and unmyelinated axons dropped at 16 weeks. But no remarkable difference was observed.

**DISCUSSION**

Nowadays, the peripheral nerve tubulization repair has been studied experimentally and clinically by different techniques. Autogenous vein graft, with or without other stuffed substances, has been utilized to repair segmental nerve tissue loss and it proved to be supportive conduits for peripheral nerve axonal regeneration and maturity. As far as the motor nerves were concerned in the previous studies, the facial nerve was not as popular as sciatic nerves from the dorsal root ganglia to be selected as the dominant test object. Actually, the facial nerve is apt to be damaged by trauma, inflammation, surgical manipulation, neoplastic diseases or other factors on account of its special location. In terms of the regeneration of damaged facial nerve, nerve segment loss causes serious consequences, such as facial paralysis and high incidence of facial synkinesis. It is necessary to find an efficient way to promote facial nerve regeneration after segmental defect and avoid the complications after injury.

It is well known that the facial nerves belong to cranial nerves. However, the peripheral axon is ensheathed by Schwann cells, which differs from glial cells environment of central nervous system (CNS), so facial nerve has greater regeneration capability than CNS. According to the composition of nerve fibers, the sciatic nerve is mixed motor nerve with a small amount of sensory fibers. On the contrary, the branches of facial nerve, distal to the parotid gland, are pure motor fibers. The study of Lloyd indicated that the motor...
nerves had better regeneration capability than sensory nerve. So we conclude that there may be some differences between facial nerve and sciatic nerve in regeneration process, by means of the autogenous standard vein grafts or inside-out vein grafts as nerve conduits.

Wang7 pointed out that inside-out vein graft has some advantages over its standard counterpart in structure and function. On the contrary, Kelleher4 thought that the inside-out technique offered no functional benefit over standard vein grafts. The former declared that the conduction velocities in the two techniques had no significant difference at 8 weeks after surgery. The latter just performed the electrophysiological assessment once three months after repair and did not prove the changes during the regeneration process. The conduit technique offers many benefits for the regeneration of peripheral nerve segment defect.16 As for our experimental result, it demonstrated that the IOVG was superior to the SVG in the 4 to 8 weeks because the IOVG produced faster nerve regeneration and earlier myelination during this early regeneration stage. Moreover, the regenerated axons in the IOVG group were closer to the normal axons although they were still smaller than the normal. It may be due to that the inside-out vein graft not only provided rich basal membrane, collagen, laminin existing in the vein wall, but also made the Schwann cells, which were located in the external coat of the vein membrane, have direct attachment with the new-born axon. On the other side, it seemed that our results about the facial nerve function recovery were more positive than that of sciatic nerve. This may be due to that the motor nerves own larger axons and more sensitive Schwann cells, which can enhance the materials exchange and accelerate the regeneration process. But it is not easy to compare the function directly between these two nerves for the lack of suitable assessment standards.

In our study, we utilized the rabbit facial vein as the nerve conduit. This vein graft is located outside the parotid gland posterior lower pole and can be easily obtained in the same incision of nerve operation without opening the parotid gland capsule; otherwise, it may increase the scar and salivary fistula morbidity. At the same time, we were satisfied with the facial vein graft, straight and without valves. Furthermore, its diameter is about 1.5-2.0 mm, just fitting for the buccal branch of facial nerve and no compression appeared to the new regenerated axon. In the study, we did not observe facial synkinesis in the experimental rabbits. It is known that ephaptic transmission between adjacent axons, hyperexcitability within facial nucleus and loss of somatotopic ability of facial nerve nucleus are possible factors of facial synkinesis.17,18 Previous works demonstrated that the tabulation technique may lessen the synkinesis by making the axon prolong following the neurotrophic factors and establish proper connections.19,20

In conclusion, inside-out vein grafts are applicable in some segmental facial nerve loss cases.

REFERENCES


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