Original Article
The role of great auricular-facial nerve neurorrhaphy in facial nerve damage

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Abstract: Background: Facial nerve is easy to be damaged, and there are many reconstructive methods for facial nerve reconstructive, such as facial nerve end to end anastomosis, the great auricular nerve graft, the sural nerve graft, or hypoglossal-facial nerve anastomosis. However, there is still little study about great auricular-facial nerve neurorrhaphy. The aim of the present study was to identify the role of great auricular-facial nerve neurorrhaphy and the mechanism. Methods: Rat models of facial nerve cut (FC), facial nerve end to end anastomosis (FF), facial-great auricular neurorrhaphy (FG), and control (Ctrl) were established. Apex nasi amesiality observation, electrophysiology and immunofluorescence assays were employed to investigate the function and mechanism. Results: In apex nasi amesiality observation, it was found apex nasi amesiality of FG group was partly recovered. Additionally, electrophysiology and immunofluorescence assays revealed that facial-great auricular neurorrhaphy could transfer nerve impulse and express AChR which was better than facial nerve cut and worse than facial nerve end to end anastomosis. Conclusions: The present study indicated that great auricular-facial nerve neurorrhaphy is a substantial solution for facial lesion repair, as it is efficiently preventing facial muscles atrophy by generating neurotransmitter like ACh.

Keywords: Facial nerve, great auricular nerve, nerve neurorrhaphy, AChR

Introduction

Due to the superficial position, facial nerve is easy to be damaged by trauma, inflammation and surgery [1]. And facial nerve lesions always combine with a significant loss of function, such as corneal exposure, epiphora, and brow ptosis, as well as external nasal valve collapse, oral incompetence, and loss of smile ability, which will lead to devastating consequences for patients [2]. Thus, the improvement of facial nerve function after injury is of the utmost importance to both patients and the physicians who treat them. Although many strategies have been used to improve the recovery of facial function after facial nerve damage like neurotrophic factors, electrical stimulation, and stem cells, peripheral motor nerve regeneration and functional recovery in humans is not robust, due to that facial nerve lesion lacks of valid spontaneous regeneration in the distal nerve stump in certain situations. Thus, nerve end to end anastomosis and graft is becoming one of the treatment guidelines for facial nerve neurotmesis.

Nerve grafts, which remove nerve segments from other part of the body, are used most commonly in facial nerve repair. In the past twenty years, most clinicians utilized accessory nerve, lingual nerve, sural nerve and great auricular nerve for facial nerve injury repair [3-5]. Unfortunately, in some cases, patients with severe and complicated facial nerve damage did not have the condition for grafts or end to end anastomosis, because of complicated anatomy of temporal bones, hardly exposed of proximal facial nerve and the severity condition of patients who couldn’t undergo a so complicated and time-long surgery [6]. In these respects, facial nerve foster with other nerves was becoming an alternative solution to delay the amyotrophy of these patients. Great auricular nerve, compared with other nerves, is neighborhood with facial nerve in anatomical position which provides great auricular-facial nerve neu-
auricular-facial nerve neurorrhaphy within a smaller surgical filed in facial nerve repair surgery. What’s more, their section areas of great auricular nerve and facial nerve are similar which may lead to the facial-great auricular nerve neurorrhaphy more efficiently than other potentially replaceable nerves. In virtue of these advantages, Great auricular-facial nerve neurorrhaphy attracted increas-

ingly attention to the surgeon.

However, there is few published report about whether the mechanism of functional recovery, nerve regeneration and innervation of great auricular-facial nerve neurorrhaphy in facial nerve repair has a connection with acetylcholine receptors (AChR) and neuromuscular junction (NMJ). As in recent presented studies, it was well recognized that maintenance of a high density of AChRs at the postsynaptic membrane of NMJ is essential for the effectiveness of synaptic impulse transmission [7]. Neuromuscular junctions are assembled on the muscle fibers at very precise locations called end plates (EP) where AChR is required for an accurate synaptic transmission.

In our present studies, we established a rat model for facial nerve injury and facial-great auricular neurorrhaphy for the first time. Then, we observed the whiskers behavior of the rats after the operation. Furthermore, muscle action potentials and AChR were detected by electrophysiology and immunofluorescence to measure the functional recovery and the nerve regeneration about great auricular nerve graft in facial nerve repair.

Materials and methods

Experimental animals and groups

The study was approved by the animal care commit of Shandong University. Eighty Wistar rats, 5~6 weeks old and weighting 200 g~220 g each, were obtained from the experimental animal centre of Shandong University. The rats were randomly assigned to 4 experimental groups (n = 20). All the objects were fed with water and standard laboratory animal chow and live libitum in a 12:12 h light-dark cycle, 21°C, environment. All procedures were approved by the Experimental Animal Administration Committee of Shandong University.

Surgical procedure

Before the procedure, all Wistar rats were anesthetized by intraperitoneal injection of 10% sodium chloral hydrate (10 ml/100 g). Then, the rats underwent a postauricular lesion in the left facial. In the next moment, the facial nerve trunk was widely exposed in the back of meatal cartilage and the facial nerve on the left side was cut where posterior auricular nerve was emerged. Great auricular nerve was cut after being separated between self-holding trapezius muscle and acromial bone trapezius muscle. In the facial nerve cut group (FC), 3 mm facial nerve trunk was cut. In the facial nerve-facial nerve end to end anastomosis group (FF), distal facial nerve section was sutured with proximal facial nerve section. In the facial-great auricular neurorrhaphy group (FG), distal facial nerve section was sutured with proximal great auricular nerve section. Finally, all the neurorrhaphy in the above 3 groups were covered with 7 mm great saphenous vein which obtained from the left leg. In the control group (Ctrl), the facial nerve was only exposed and remained intact.

Apex nasi amesiality observation and electrophysiology

To investigate whether the saturation could improve the physiological function of facial nerve injury, Apex nasi amesiality observation...
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was utilized. In the 8 and 12 weeks after the operation, apex nasi amesiality behaviors of each group rats' were observed.

Then, each of the ten rats was anesthetized as described above. The saturation of facial nerve was re-exposed to ensure the saturation was succeeded. Subsequently, buccal surface branch of the facial nerve was further separated to evaluate the function of facial nerve after the suturation. The stimulating electrode was inserted in the cross of the canthus vertical line and the buccal surface branch of the facial nerve, and the collecting electrode was inserted subcutaneously at the left side of orbicularis oris muscle (Figure 1). Electrophysiology was performed with multichannel Medelec Synergy electrophysiological instrument (Oxford Instruments Medical, Oxford, England) and a stimulation of 1.0 mV was forced. Incubation time and amplitude of vibration was recorded.

Histology sections and immunofluorescence

At the end of the study, all rats were euthanized by an overdose of 4% paraformaldehyde via intracardiac injection. The left orbicularis oris muscles of the rats were immediately harvested, pinned to resting length, fixed with 4% paraformaldehyde at 4°C for 12 h, and immersed in isopentane cooled with dry ice. The muscles were sectioned on a cryotome at 8 um thick and stored at -80°C.

The sections were rinsed at room temperature using 0.01 M phosphate buffered solution (PBS) for three times (5 min per time). Subsequently, sections were permeabilized with 0.1% Triton X-100 for 20 minutes, and then incubated with AlexaFluor 488-conjugated α-bungarotoxin (1:200; Invitrogen, Carlsbad, California) in dark chamber for 1 h. After washing with PBS for 15 min, Confocal images microscope (Lecia, German) was employed to observe the fluorescence of the AChR.

Statistical analysis

Data were presented as mean ± standard deviation. Statistical calculations were performed by SPSS 13.0 software package (SPSS Inc, Chicago, IL, USA). One-way analysis of variance and least significance divergence were applied to analyze the data. P values lower than 0.05 (two-tailed) were considered significant.

Results

Apex nasi amesiality

As shown in Figure 2, in the eighth week, the Ctrl group rats have no apex nasi amesiality, while, the other three groups have obvious apex nasi right amesiality. In 12th week, rats of Ctrl group still keep normal and have no apex nasi amesiality. In FF group, rats' noses are right amesiality lightly, which is much better than that in the 8th week. In contrast, Apex nasies of

Figure 2. Apex nasi amesiality of each group in 8 weeks and 12 weeks after operation.
FG group still have an apparent right amesiality, though that is a little better than that in the 8th week. In FC group, rats’ apex nasi right amesiality as well as in 8 week.

**Electrophysiology**

As shown in Figure 3, eight weeks after operation, latency of the FF group (2.413±0.479 ms) and FG group (3.298±0.383 ms) markedly prolonged as compared with that of the Ctrl group (1.534±0.319 ms), and the peak-to-peak amplitudes of the FF group (0.903±0.264 mV) and FG group (0.489±0.044 mV) were obviously than that of the Ctrl group (1.829±0.054 mV). At the twelfth week, latency of FF group, FG group and Ctrl group were 1.827±0.335 ms, 3.373±0.308 ms, 1.078±0.109 ms. The peak-to-peak amplitudes of the three groups were 1.525±0.138 mV, 0.758±0.146 mV, 1.889±0.102 mV, respectively. Obviously, latency and the peak-to-peak amplitude FF group have an evident improvement in 12 weeks as compared with that in 8 weeks. Though FG group also had a more apparent improvement in 12 weeks than in 8 weeks, their function was still far from FF groups. In addition, FC group, the amplitude was no elicitation no matter in 8 weeks or 12 weeks.

**Immunofluorescence of AChR expression**

AChRs were stained with AlexaFluor 488-conjugated α-bungarotoxin and observed using laser scanning confocal microscopy. As shown in Figure 4, in the week 8, AChRs were highly contiguous, complex, and had deep gutters in Ctrl group. In FF group, AChRs were expressed less weakly than that of Ctrl group. In contrast, there was only a very weak expression of AChRs in FG group, while we detected little fluorescence of AChRs in FC group. In Week 12, no obvious of AChRs expression was observed in comparison with FF group and Ctrl group. In FG group, AChRs expression was improved compared with that in week 8 and still had an evident difference with Ctrl and FF group. There was still no obvious fluorescence of FC group in week 12 as well as that in week 8.

**Discussion**

In the recent study, it was well recognized that surgery is clearly superior to offering no treat-
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In nerve lesions, end to end anastomosis is the main approach [8, 9]. To investigate the value of great auricular-facial nerve neurorrhaphy in facial nerve neurotmesis, in the present study, a rat model of facial cut injury was established primarily for recognizing the differences of facial movement recovery between facial nerve end to end anastomosis and great auricular-facial nerve neurorrhaphy. As expected, facial nerve end to end anastomosis had a better performance in apex nasi amesiality recovery than that of great auricular-facial nerve neurorrhaphy. As a mixed nerve, facial nerve injury led to the movement, feeling, and nutrition disorder in its innervation area. Thus, end to end anastomosis is the prior principle of facial nerve injury. However, in cases of facial nerve cut injury, end to end anastomosis must reroute the facial nerve as the nerve stump tensioning present, or hardly be adopted as facial nerve damaged seriously [9, 10]. In this way, great auricular-facial nerve neurorrhaphy could be employed as one-stage surgery or for primary function recovery. Great auricular nerve, a branch of the superficial cervical plexus, which is neighbored to the facial nerve and has a similar cross-sectional trunk area with facial nerve as we illustrated above is becoming a common choice in facial nerve repair. In our study, it has been found that great auricular-facial nerve neurorrhaphy is partly apex nasi amesiality recovery which is better than facial cut without repair.

Furthermore, in electrophysiology, end to end anastomosis was latency shorter and amplitudes deeper than that of great auricular-facial nerve neurorrhaphy, which is approximate with the function recovery. Since muscle movement has been associated with AChR expression and NMJ size [11], we suspected that great auricular nerve possibly could generate neurotransmitter in the NMJ to prevent the atrophy of muscles which innervated by facial nerve, though, as a sensory nerve, it is disabled in innervating muscles movements.

To further investigate our conjecture, we analyzed the AChR expression in the NMJ. NMJ is composed of three components: the nerve terminal, the muscle fiber, and the schwann cell [12]. Synapse, which transmits electrical impulses from the nerve terminal to the skeletal muscle via the chemical transmitter, acetylcholine (ACh), has three major structural elements: the presynaptic region containing the nerve terminal; the synaptic cleft; and the postsynaptic surface containing AChR which induces the endplate potential (EPP) [13]. Normally, the nerve terminal could release enough ACh for initiating an action potential. However, abundances of Ach molecules are only one of the factors to promote consistent communication between nerve and muscle. Another factor is acetylcholinesterase (AChE), which is anchored in the basal lamina, and whose activation is along with the diffusion of Ach. The activation of AChE hydrolyzes Ach and prevents the initiat-
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In the NMJ, the action of Ach and the inhibition of AChE activity would enhance the activation of AChR, which permits passage of cation through and produces nerve and muscle communication [15]. Thus, at a functional standpoint, AChR is the most important protein in the NMJ and has been the subject of muscle movements. In our present study, it was observed that AChR was expressed on the NMJ in FF group, partly expressed of FG group, little expressed in FC Group, in contrast with Ctrl group. Based on this phenomenon, we inferred that great auricular-facial nerve neurorrhaphy would generate Ach and partly activate AChR in the NMJ, which prevented the muscle atrophy and had led to an action potential when a stimulating was given by electrode in vitro. This is in accordance with our conjecture. However, great auricular nerve, as a sensory nerve, would never generate a nervous impulse autonomously to modulate muscle movement. So great auricular-facial nerve neurorrhaphy in facial nerve repair could not promote the movements of facial muscles, it has only improved the situation of facial muscle amyotrophy by generated neurotransmitter such as Ach.

In addition, what attracted our attention is that all the results of FG group and FF group in the 12th week were much better than that in the 8th week which indicated that the recovery of facial nerve lesion need 8 weeks or longer time. In this way, our present work would have a more desired result if we prolonged our study time.

In summary, our experiments have demonstrated that great auricular-facial nerve neurorrhaphy and facial nerve end to end anastomosis are all adequate solutions for the repair of facial nerve lesions. Great auricular-facial nerve neurorrhaphy is efficiently preventing facial muscles atrophy by generating neurotransmitter like Ach, but never promoting the movements of facial muscles. So great auricular-facial nerve neurorrhaphy is only a potential solution for facial nerve repair which should be preferred in those that end to end anastomosis is hardly accepted as facial nerve is badly destroyed.

Disclosure of conflict of interest

None.

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References

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