Improved C\textsubscript{3-4} transfer for treatment of root avulsion of the brachial plexus upper trunk

\textit{Animal experiments and clinical application}\textsuperscript{***☆}

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Abstract
Experimental rats with root avulsion of the brachial plexus upper trunk were treated with the improved C\textsubscript{3-4} transfer for neurotization of C\textsubscript{5-6}. Results showed that Terzis grooming test scores were significantly increased at 6 months after treatment, the latency of C\textsubscript{5-6} motor evoked potential was gradually shortened, and the amplitude was gradually increased. The rate of C\textsubscript{3} instead of C\textsubscript{5} and the C\textsubscript{4} + phrenic nerve instead of C\textsubscript{6} myelinated nerve fibers crossing through the anastomotic stoma was approximately 80%. Myelinated nerve fibers were arranged loosely but the thickness of the myelin sheath was similar to that of the healthy side. In clinical applications, 39 patients with root avulsion of the brachial plexus upper trunk were followed for 6 months to 4.5 years after treatment using the improved C\textsubscript{3} instead of C\textsubscript{5} nerve root transfer and C\textsubscript{4} nerve root and phrenic nerve instead of C\textsubscript{6} nerve root transfer. Results showed that the strength of the brachial biceps and deltoid muscles recovered to level III–IV, scapular muscle to level III–IV, latissimus dorsi and pectoralis major muscles to above level III, and the brachial triceps muscle to level 0–III. Results showed that the improved C\textsubscript{3-4} transfer for root avulsion of the brachial plexus upper trunk in animal models is similar to clinical findings and that C\textsubscript{3-4} and the phrenic nerve transfer for neurotization of C\textsubscript{5-6} can innervate the avulsed brachial plexus upper trunk and promote the recovery of nerve function in the upper extremity.

Key Words
brachial plexus; cervical plexus; upper trunk; root avulsion; nerve transfer; phrenic nerve; translational medicine; peripheral nerve injury; neural regeneration

Research Highlights
(1) Rats with root avulsion of brachial plexus upper trunk were successfully treated with the improved C\textsubscript{3-4} nerve transfer and results were confirmed with clinical studies. (2) C\textsubscript{3} and C\textsubscript{4} + phrenic nerve transfer for neurotization of C\textsubscript{5-6} can innervate the avulsed brachial plexus upper trunk and promote the recovery of nerve function in the upper extremity.
INTRODUCTION

Brachial plexus injury is a common form of peripheral nerve injury. Root avulsion is the most serious, with the highest incidence in the middle and upper trunks. Current treatment involves nerve transfer within the plexus or surrounding the plexus[1-8]. However, lack of nerve matching between recipients and donors and excessive length of grafted segments make these methods unsatisfactory. It is critical to choose a strongly regenerative nerve as a donor without impacting the original nerve function.

In 1991, Yamada et al[9] used C3-4 nerve root transfer to repair injury to the C5-6 nerve root, and found that the strength of the surrounding muscle in the shoulder girdle and biceps brachii recovered to level IV. Cao et al[10] found that it is feasible to treat brachial plexus upper trunk avulsion in New Zealand white rabbits using C3-4 nerve transfer, and presented the concept of "root repair" in 2001. In the above two studies, the ipsilateral phrenic nerve was preserved in both human patients and animal experiments. We hypothesized that the phrenic nerve can be transposed to the C6 nerve root or upper trunk in combination with C3-4 nerve transfer to increase the number of transferred nerves and promote functional recovery.

This study aimed to perform both C3-4 nerve transfer and phrenic nerve transposition to the upper nerve trunk in Wistar rats to provide experimental evidence for improved C3-4 nerve transfer treatment of brachial plexus upper trunk avulsion. Based on animal experiments, 39 patients were then treated.

RESULTS

Animal experiments

Quantitative analysis of experimental animals

Thirty rats, divided into two groups, were included to establish a right brachial plexus upper trunk avulsion model. Experimental group (n = 20): rats were treated with the improved C3-4 instead of C5-6 nerve transfer method. Model control group (n = 10): the right C5-6 nerve was avulsed and the uninjured side of the experimental group served as normal controls. There was one unexplained death in the experimental group and data from 29 rats were involved in the analysis.

General condition of rats and gross observation at injury site

There was no swelling, necrosis or exfoliation in the forelimb of the operated side in all rats before surgery; neither plantar ulcers nor hindlimb paralysis occurred. All rats showed paralysis of the contralateral shoulder and elbow after operation, and muscle atrophy in the operated arms was visible over time. The difference was not observable with the naked eye. No dehiscence was observed at the nerve anastomosis site based on anatomical observations in the experimental group. The bundle branches at the proximal nerve anastomosis site became one nerve bundle. At 3 months post-surgery, the anastomosis site showed significant adhesions and rough nerve surfaces, with a smooth appearance at the distal anastomosis site. At 6 months post-surgery, the nerve anastomosis site had only mild adhesions and a smooth appearance, with improved appearance of the distal nerves. The model control group showed severe perineural adhesions and cord-like scars of the nerve root.

Improved C3-4 nerve transfer technique ameliorated nerve function in rats with right brachial plexus upper trunk avulsion

Results of the Terzis grooming test showed that rats in the experimental group scored increasingly better over time, while the model control group scored 0 (supplementary Figure 1 online, Table 1).

Improved C3-4 nerve transfer technique improved electrophysiological indices in rats with right brachial plexus upper trunk avulsion

At 3 and 6 months post-surgery, C5-6 evoked potential latency and amplitude were recorded using electromyography. The latency of motor evoked potentials was gradually shortened while the amplitude was gradually increased in the experimental group over time (P < 0.05). At 6 months post-surgery, there was no significant difference in the C6 evoked potential latency and C2 amplitude between the experimental and normal control groups (P > 0.05). In the model control group, no evoked potentials were found (Table 2).

Pathological changes of injured tissue in rats with
right brachial plexus upper trunk avulsion

Hematoxylin-eosin staining and counting results showed that nearly 80% of myelinated nerve fibers crossed the anastomosis site, regardless of whether C3 or C4 + phrenic nerve transfer was used. There were significant differences compared with normal C5 and C6 nerves, accounting for 29.1% of normal C5 through the myelinated nerve fibers in C5 and 61.5% of normal C6 through the myelinated nerve fibers in C6 (Figure 1). Statistical analysis results demonstrated no significant difference in the number of myelinated nerve fibers between C5 and C3 in the experimental group at 3 and 6 months post-surgery (P > 0.05), while the number was decreased significantly compared with normal C5 myelinated nerve fibers (P < 0.05). A similar change was observed at C6 in comparison to normal C4 + phrenic nerve and normal C6 (P < 0.05). But the numbers of C5 and C6 myelinated nerve fibers were the same in the experimental group at 3 and 6 months (P > 0.05; Table 3).

Nerve fiber ultrastructure of injured tissue in rats with right brachial plexus upper trunk avulsion

Under transmission electron microscopy, normal myelinated nerve fibers were closely and clearly arranged, and the myelin arrangement became slightly loose at 3 and 6 months after C3 and C4 + phrenic nerve transfer in the experimental group (Figure 2). There was no significant difference in the myelin thickness among groups (P > 0.05; Table 4).

Clinical study
Quantitative analysis of subjects

A total of 39 patients were included in this study and followed up for 6 months to 4.5 years. All 39 patients were involved in the result analysis.

Table 2  Change of motor evoked potential latency (ms) and amplitude (mV) of rats in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Time after surgery (month)</th>
<th>n</th>
<th>Latency C5</th>
<th>Latency C6</th>
<th>Amplitude C5</th>
<th>Amplitude C6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>3</td>
<td>10</td>
<td>1.28±0.14</td>
<td>1.37±0.29a</td>
<td>11.91±1.49ab</td>
<td>11.16±1.63ab</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>9</td>
<td>1.15±0.17a</td>
<td>1.26±0.14</td>
<td>14.20±1.84</td>
<td>14.70±1.36a</td>
</tr>
<tr>
<td>Model control</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Normal control</td>
<td>10</td>
<td>0</td>
<td>0.98±0.13</td>
<td>1.07±0.17</td>
<td>15.78±1.93</td>
<td>17.07±1.72</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SD; n refers to the number of rats in each group at each time point. *P < 0.05, vs. normal control group; **P < 0.05, vs. 6 months in experimental group (one-way analysis of variance).

Figure 1  Morphology of C5,6 myelinated nerve fibers in rats of each group (hematoxylin-eosin staining, light microscope, × 400).
(A) C5 in normal control group; (B) C5 in experimental group at 3 months; (C) C5 in experimental group at 6 months; (D) C4 in normal control group; (E) C3 in experimental group at 3 months; (F) C4 in experimental group at 6 months; (G) C5 in normal control group; (H) C6 in normal control group.
Neural functional recovery of involved subjects

During the follow-up period, the sensory function of most patients recovered and the strength of the brachial biceps and deltoid muscles were level III–IV in most patients. The strength of the scapular muscles was level III–IV, latissimus dorsi muscle and pectoral muscle was level III or higher, and the triceps was level 0–IV (due to postoperative time differences, improvement of motor function is displayed in further follow-ups; Table 5).

Three cases were followed for 4 years post-surgery with brachial biceps and deltoid muscles assessed as level IV–V and triceps strength as level 0–IV (one patient did not recover in muscle strength after surgery but could extend the elbow following functional reconstruction surgery).

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[Table 3]

<table>
<thead>
<tr>
<th>Group</th>
<th>Time after surgery (month)</th>
<th>n</th>
<th>C3</th>
<th>C4+phrenic nerve</th>
<th>C5</th>
<th>C6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>3</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>260.80±68.67a</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>278.78±55.99a</td>
</tr>
<tr>
<td>Model control</td>
<td>3</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Normal control</td>
<td>10</td>
<td>313.90±52.39</td>
<td>928.30±56.43</td>
<td>894.30±54.42</td>
<td>198.40±75.58</td>
<td></td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SD; n refers to the number of rats in each group at each time point. *P < 0.05, vs. normal control group; **P < 0.05, vs. C4 + phrenic nerve in normal control group (one-way analysis of variance).
Typical case

A 33-year-old, male patient was admitted to the hospital in September 2003 for right shoulder and right upper arm sensory and motor impairments due to a mechanical stretch injury which occurred 3 months previously. Physical examination revealed right upper extremity overhang, loss of right upper arm skin sensation, muscle strength level 0, pectoralis major and latissimus dorsi muscle strength level 0. Right upper brachial plexus complete injury was confirmed by electromyogram (Figure 3). Surgical investigation showed a C5-6 root avulsion in the right arm with the proximal end not seen until the intervertebral foramen. C3-4 was separated to the distal end along each nerve branch and the muscle was transected, freeing the phrenic nerve and allowing direct anastomosis with the torn ends of C6. The sural nerve was isolated for graft bridging at C3-5 and C4-6 and the phrenic nerve stump was anastomosed with C6 (supplementary Figure 2 online). At 6 months post-surgery, the patient felt coarse skin sensations in the right upper arm and complained about right upper limb abduction (muscle strength level II). At 14 months post-surgery, skin sensation in the right upper arm was restored, and the right shoulder showed 45° abduction and 30° anterior flexion. Right biceps brachii muscle contraction was palpable, but the elbow joint could not flex (muscle strength level I). At 18 months post-surgery, the right biceps brachii and deltoid muscles recovered to level 0 muscle strength (supplementary Figure 3 online), scapular peripheral muscle strength recovered to level IV–V, the elbow joint could flex normally, shoulder abduction, flexion and adduction were normal, and latissimus dorsi and pectoral muscle strength recovered to level IV–V. However, triceps brachii muscle strength was not restored. Electromyography displayed some regenerative potentials in the right triceps brachii, but no action potential was seen. Latissimus dorsi muscle flap transposition was performed to reconstruct elbow extension function in December 2006, and the patient recovered normal function of the right upper extremity at 1 year post-surgery (supplementary Figure 4 online; Figures 4, 5).

Table 4 Thickness of myelinated nerve fibers (μm) in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>C3</th>
<th>C4+ phrenic nerve</th>
<th>C4</th>
<th>C5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>3 months</td>
<td>10</td>
<td>–</td>
<td>–</td>
<td>0.58±0.16</td>
</tr>
<tr>
<td>Model control</td>
<td>3 months</td>
<td>5</td>
<td>–</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>Experimental</td>
<td>6 months</td>
<td>9</td>
<td>–</td>
<td>–</td>
<td>0.59±0.14</td>
</tr>
<tr>
<td>Model control</td>
<td>6 months</td>
<td>5</td>
<td>–</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>Normal control</td>
<td>9 months</td>
<td>10</td>
<td>0.61±0.12</td>
<td>0.62±0.14</td>
<td>0.61±0.13</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SD; n refers to the number of rats in each group at each time point. One-way analysis of variance showed no significant difference in C5-6 myelin thickness of rats in the experimental group at 3 and 6 months, compared with normal C3-6 (P > 0.05).

Table 5 Recovery of muscle strength in patients from follow-ups to July 2010 (n)

<table>
<thead>
<tr>
<th>Strength</th>
<th>0</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biceps brachii</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>19</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>Deltoid muscle</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>Scapular muscles</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>Latissimus dorsi muscle and pectoral muscle</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>26</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Triceps brachii</td>
<td>2</td>
<td>3</td>
<td>19</td>
<td>13</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 3 Axillary nerve (upper) and musculocutaneous nerve (lower) motor evoked potentials before (A) and 1 year after surgery (B).

Figure 4 Axillary nerve (A) and musculocutaneous nerve (B) motor evoked potentials at 1.5 years post-surgery.
DISCUSSION

There are few studies reporting C3-4 nerve transfer instead of C5-6 nerve transfer in rat models. Cao et al. [10] used C3-4 nerve transfer in the treatment of brachial plexus upper trunk avulsion in New Zealand rabbits, but the phrenic nerve was not transposed. This study adopted animal models based on studies by Yamada et al. [9] and Cao et al. [10], with the following improvements:

1. Increase the direct anastomosis between the phrenic nerve and C6. The phrenic nerve is a motor nerve and phrenic nerve transfer was used as a therapy for brachial plexus root avulsion, as reported by Gu et al. [2] since 1989. This nerve remains an important dynamic nerve source. Among the existing repair methods for brachial plexus injury, phrenic nerve repair of the musculocutaneous nerve achieves the best results. On one hand, the musculocutaneous nerve-dominated brachial biceps muscle fibers are thick and located proximally, requiring a short time for neural regeneration to the muscles. On the other hand, the phrenic nerve is abundant in motion nerve fibers and possesses spontaneous electrical impulses, which promotes neural regeneration. In addition, elbow flexion is the most important function of the upper limb in total brachial plexus injury patients, so phrenic nerve transfer is the preferred surgical approach for the repair of the musculocutaneous nerve. However, phrenic nerve and musculocutaneous nerve anastomosis requires neural transplantation, and transplantation of free nerves over 10 cm in length is prone to denaturation and yields poor results due to an absence of blood vessels. Such operations can waste motor nerve sources that are already limited in the neck, and cause a loss of power nerves, which is not conducive to functional recovery. The cervical plexus motor branch mainly innervates tiny cervical muscles, such as the sternocleidomastoid and trapezius muscles, which have smaller fibers than the deltoid and biceps brachii muscles in the upper trunk of the brachial plexus. In this study, the phrenic nerve was isolated and directly anastomosed with the C6 nerve root, removing the need for neural transplantation. In combination with C2 nerve root transfer to repair the C6 nerve root, the phrenic nerve may play an auxiliary role in the functional recovery of C6 nerve roots due to its spontaneous electrical impulses. The total number of human phrenic nerve fibers is 2,700, which is more than that of the accessory nerve (2,200), cervical nerve motor branch (900), and the intercostal 4–5 root (630–700). Furthermore, the phrenic nerve consists of motor nerve fibers, which can significantly improve motor nerve fiber content in the C6 nerve root. Results showed that biceps brachii function was well recovered. In this study, the C6 nerve root showed the greatest percentage of myelinated nerve fibers (65.2% of normal C6), indicating the necessity of adding the phrenic nerve.

2. Shorten the nerve graft length and increase the graft number: in this study, C3-4 anatomical separation and transection was not performed in a single cutting fashion. Instead, we separated the main branches to the most distal point and followed with muscle transfer. Although the isolated cervical plexus branches were uneven, each branch could be directly anastomosed to the ends of C5-6, which removed the need for neural transplantation. Once a free nerve graft exceeds a certain length, it is prone to degeneration and some nerve fibers were lost, slowing nerve growth and hindering functional recovery. A direct end-to-end anastomosis between C3-4 nerve branches and C5-6 nerves can save an anastomotic stoma and it is not necessary to cross through the avascular nerve grafts protecting the motor nerve source, promoting nerve growth and functional recovery. In addition, this operation does not separate nerves for transplantation, which reduce surgical trauma in experimental animals, benefits intraoperative and postoperative survival, and reduces animal death.

It is common to separate C6 and the phrenic nerve, neatly cut the distal ends, and choose 2–3 branches of the sural nerve for cable grafts. Each branch is isolated to the distal end so even very short segments, sometimes only 1 cm in cases of fresh trauma, can be directly sutured. Although the nerve bundles for neural transplantation are of uneven length, the length of the nerve graft is shortened, decreasing problems during regeneration, shortening nerve grafting time, and improving the number of nerves reaching the effective axons at the distal end. One-by-one transplantation or two smaller,
combined transplants require more allon graft nerve segments, usually 4–6 branches. The required graft is short, making the sural nerve a suitable choice. A large number of branches allows more pathways for nerve growth toward the distal end.

(3) Implement fine repair. The recovery of motor function is more important than sensory function in the superior brachial plexus. The cervical plexus contains large numbers of fibers, including 4 090 motor fibers and 3 250 sensory fibers. After overall resection and cable grafting, it is difficult to distinguish sensory nerve and motor nerve branches, and to perform end-graft and repair. During axonal regeneration, both sensory and motor nerves grow into the upper trunk of the brachial plexus through the nerve graft. Nerve growth in the upper trunk of the brachial plexus is decreased after injury and a large number of sensory nerve fibers invade the space for motor nerve growth. This interferes with motor neuron growth into the distal C 3-4 nerve root, or dislocates to the end effector (motor endplate). Sensory nerves grow into motor nerves and begin to degenerate after reaching the end effector, blocking motor nerve pathways and affecting the recovery of motor function. In this study, the muscle branch was separated and anastomosed with C 5-6. Fine transplantation may decrease competitive growth of sensory nerve fibers in nerve repair processes, thereby reducing the extrusion and inhibition effects of sensory nerves on motor nerve growth, ensuring motor nerve fibers connect with the distal nerve root, allowing for better functioning of target organs and effectors, and promoting functional recovery. Notably, C 5-6 nerve fiber myelin was thickened in some areas in the experimental group, involving up to one-third of the myelin thickness. This finding requires further studies.

After nerve transposition for the repair of brachial plexus root avulsion, both the avulsed nerve root and the nerve transfer anastomosis act as peripheral nerve injuries. Following peripheral nerve injury, Wallerian degeneration occurs readily with the loss of the nutritional support of the neuronal cell bodies, so the distal nerve fiber is bound to degenerate. Clinically, brachial plexus root avulsion is surgically treated 3 months or longer after injury and by this time, Wallerian degeneration is evident in the avulsed nerve root, the endoneurial tube is completely collapsed and actual disappearance of the endoneurial tube and Schwann cells can be seen. In such cases, nerve transposition cannot induce neural regeneration in a short recovery time. This delay in repair explains why animal experiments are better than clinical studies, and why neural regeneration in rats is faster than in the human.

The experimental animal model established in this study suggested a promotion of neural regeneration, but these findings do not yet match clinical practice. Improved results in the experimental model can be due partly to the reduced time to surgical repair of the brachial plexus root avulsion, and the reduced number of anastomotic stoma due to no nerve grafting. Repair immediately after avulsion cannot only reduce Wallerian degeneration, but also protect the spinal cord anterior horn motor neurons. The present experiments showed that nearly 80% of myelinated nerve fibers crossed through the anastomosis in both C 3 and C 4 + phrenic nerve methods. In addition to the loss of some myelinated nerve fibers, the apoptosis of spinal anterior horn motor neurons also contributes to the reduced percentage, but the effect was significantly decreased. This model also indicates the necessity of early surgery in treatment of brachial plexus root avulsion.

Recovery of muscle function requires reinnervation. Motor nerve axons must connect with the target muscle motor endplate or generate new motor endplates and the connection should be strong enough to cause muscle contraction and generate normal muscle function. The motor endplate is the terminal structure of motor nerve fibers and post-injury changes are similar to the distal end after nerve trunk resection. The injured nerve atrophies and the degree of atrophy worsens with time. Once muscle nutrition disappears, muscle atrophy is inevitable. Muscle cells begin to change 3–4 months after injury and changes progress over approximately 2 years. Muscle fibers disintegrate and are replaced by connective tissue while nerve axons grow but cannot form the endplate on connective tissue. As a result, it is not possible to regenerate nerve function longer than 2 years following injury.

The Terzis grooming test describes and assesses behaviors and actions and is usually used in neural system testing, demonstrating the maturation and reconstruction of the target muscle motor units after nerve reinnervation. The modified behavior of rats includes elbow flexion and shoulder abduction or lifting, which reflects the functional recovery of biceps brachii and deltoid muscles. Results of this experiment showed that muscle function was well-recovered in the experimental group at 3 and 6 months post-surgery, particularly at 6 months. However, Terzis grooming test scores were significantly different compared with the normal side. The reasons may be as follows: (1) C 3-4 nerve root and the phrenic nerve have fewer motor nerve fibers than the C 5-6 nerve root and the number of nerves connecting with the target muscle motor end-plate was lower than in normal muscle. (2) The consequences of disuse muscle atrophy were not fully corrected during the experiments. (3) The neurological remodeling process in the spinal cord and brain was incomplete, especially for the phrenic nerve. (4) The physiological and functional training were insufficient after nerve...
transfer.

Cervical plexus nerves mainly innervate the small cervical muscles and dominate their movement. C3-4 resection may induce motor dysfunction but this was not seen in the present study. The reasons may be as follows: (1) only the cervical nerve anterior branch was removed during the operations with the posterior branch unaffected. The lateral and back muscles were innervated by the posterior branch. (2) C3-4 muscular branches innervate the sternocleidomastoid, levator scapulae, trapezius, anterior scalene, middle scalene and omohyoides, sternohyoid and sternothyroid muscles, which receive more than two sources of cervical nerve innervation. Damage to C3-4, only, cannot completely eliminate the innervation to these muscles. Based on these two reasons and the animal experiments, we believe that this surgical approach has no impact on the motor function of the C3-4 neural donor area. In clinical cases, the C3-4 innervated donor area is not significantly affected although one patient complained of loss of skin sensation in the face and ears. Complications at the donor area require further studies due to the small number of cases.

Phrenic nerve transfer is a hotly debated topic, with concern regarding diaphragmatic function, lung function and complications after phrenic nerve resection on one side. According to a 2-year follow-up of 21 patients after phrenic nerve transposition for treatment of brachial plexus preganglionic injury reported by Zhang and Gu[19] in 1994, pulmonary function recovered to 85% of normal 1 month post-surgery, to over 90% at 6 months, and reached 100% at 2 years. Among 21 patients, 11 cases recovered normal function at 1 month post-surgery, 7 cases at 3 months and 3 cases at 6 months. The conclusion is that changes in pulmonary function are temporary after phrenic nerve transfer and function can recover to normal within 6 months. Fackler et al[20] reported similar results and did not find changes in respiratory function in rats. Recent animal experiments using phrenic nerve end-to-side anastomosis minimized the impact on diaphragmatic function[21].

**Methods**

Establishment of right brachial plexus upper trunk avulsion models and intervention: rats were intraperitoneally anesthetized with ketamine hydrochloride (100 mg/kg). A 4 cm long anterior cervical paramedian incision was made extending to the middle clavicle, upward along the trachea then right for a distance equal to one-third of the paramedian incision. The skin and subcutaneous tissues were removed and the platysma was cut, bluntly separated along the sternocleidomastoid muscle medial margin, with dissection continuing downward to the lateral carotid sheath, thus protecting the carotid artery and vagus nerve and exposing the brachial plexus upper trunk and C3-4 nerve root. The brachial plexus upper trunk was stripped in a retrograde direction to the C5-6 nerve root under × 6 surgical magnification (Gx-SS-ZZ-3, Shanghai Medical Optical Instrument Factory, Shanghai, China), freeing the nerve root. Along with the brachial plexus nerve roots, the phrenic nerve was set free and protected then the C5-6 nerve root was avulsed from the spinal cord using a self-made nerve hook (Figure 6).

The C3-4 nerve root was isolated to the distal end until the entry point to the muscle. The C5-6 nerve root avulsion was trimmed and the phrenic nerve was cut and directly anastomosed with the C6 nerve root using an 11-0 non-invasive suture line. The remaining C4 nerve branches were anastomosed with the C6 nerve root proximal end individually. The C3 branches were also anastomosed with the C5 nerve root proximal end individually, thus establishing animal models treated with the improved C3-4 nerve transfer for repairing brachial plexus.

**MATERIALS/SUBJECTS AND METHODS**

**Animal experiment**

**Design**

A randomized controlled animal experiment.

**Time and setting**

Experiments were performed from June 2007 to January 2009 in the Experimental Animal Center of the General Hospital of Jinan Military Command of Chinese PLA.

**Materials**

Thirty clean, healthy, adult, male Wistar rats, aged 3 months, weighing 220-250 g, were provided by the Experimental Animal Center of Shandong University School of Medicine (license No. SCXK (Lu) 20030004). All procedures were performed in accordance with the Guidance Suggestions for the Care and Use of Laboratory Animals, formulated by the Ministry of Science and Technology of China[22].

**Figure 6** The avulsed C5-6 nerve root and the anatomically separated C3-4 nerve root (long green arrow indicates C3, short green arrow C4, long black arrow C5, short black arrow C6).
upper trunk avulsion injury (Figure 7).

In the model control group, the avulsed C₅-₆ nerve root was left open after resection of some of the nerve bundles. The platysma and sternocleidomastoid muscle were sutured, and the wound was closed. Rats were subjected to natural waking and fed in separate cages at 2 weeks post-surgery, with no immobilization. Rats were intramuscularly injected with 20 000 U/d gentamycin sulfate within the first week post-surgery. The Terzis grooming test for assessment of nerve function: rats were evaluated with the Terzis grooming test at 3 and 6 months. Scoring criteria: 0: no action, 1 point: reaching the mouth and jaw for assessment, 2 points: reaching the area between the eyes and the mouth, 3 points: reaching the eyes, 4 points: reaching the area before the ears, 5 points: reaching the ears. Electrophysiology for assessment of nerve function recovery: rats were routinely anesthetized and the C₃-₄ nerve root and the C₅-₆ nerve root on the normal side were exposed. Recording electrodes were inserted into the deltoid and biceps brachii muscles. Rats in the experimental group received electrical stimulation at the C₃-₄ nerve root and C₅-₆ nerve root on the healthy side, while the model control group was electrically stimulated at the C₅-₆ nerve root, only. The latency and amplitude of evoked potentials were recorded using an electromyography/evoked potential instrument (Keypoint, Dantec, Copenhagen, Denmark). Hematoxylin-eosin staining for counting myelinated nerve fibers at the injury site: C₃-₄ and C₅-₆ nerve root fibers were harvested to prepare paraffin sections, which were then stained with hematoxylin-eosin. Specimens were examined under light microscopy (Olympus, Tokyo, Japan) and the number of myelinated nerve fibers in the nerve bundle was calculated. Transmission electron microscopic observation of the nerve ultrastructure at the injury site: the C₃-₄ nerve was anastomosed with nerve segments 0.3 cm away from the contralateral C₅-₆ nerve roots. Semi-thin sections were fixed and positioned. Nerve fiber cross-sections were cut into 60-70 nm thick slices, followed by uranium and lead double staining. Sections were examined under transmission electron microscope (JEM-1200EX type; JEOL Company, Tokyo, Japan) and the thickness of the myelin sheath was measured. Statistical analysis: data were statistically analyzed with SPSS 13.0 software (SPSS, Chicago, IL, USA) and measurement data were expressed as mean ± SD. Data in each group were compared with one-way analysis of variance and comparison of count data was performed using the sum rank test. \( P < 0.05 \) was considered statistically significant.

Clinical study

Design

A clinical observation.

Time and setting

Experiments were performed from June 2003 to December 2009 in the General Hospital of Jinan Military Command of Chinese PLA.

Subjects

A total of 39 male patients with brachial plexus upper trunk avulsion were admitted to the General Hospital of Jinan Military Command of Chinese PLA from June 2003 to December 2009. The age range was 17-59 years with an average of 32 years. Twenty-two cases resulted from traffic accidents, 11 cases from falls, and 6 from mechanical injury. Sixteen cases also included a fracture of the clavicle. The time from injury to admission ranged from 1 week to 6 months with a mean of 3 months. Preoperative physical examinations and electromyography were performed to exclude cases with brachial plexus middle and trunk injuries. All patients were repaired with C₃ nerve root transfer and C₄ nerve root + phrenic nerve transfer (sural nerve graft). All patients were diagnosed intraoperatively with brachial plexus upper trunk avulsion. All experimental disposals were in accordance with ethical requirements of the Declaration of Helsinki, and all the patients signed informed consent.

Methods

C₃ and C₄ + phrenic nerve transfer for treatment of C₅-₆ root avulsion: after patients were anesthetized, an arc incision was made along the posterior border of the sternocleidomastoid muscle, then skin, subcutaneous
tissue and platysma were excised exposing the fat layer. The transverse cervical artery was then ligated. The phrenic nerve was placed in the anterior scalene muscle, and the C_{4,5} nerve root was also repositioned. Each branch extending from the C_{4} nerve root was carefully separated, particularly at the distal end. In general, the length of the phrenic nerve should be directly anastomosed with the distal end of the C_{5} nerve root. Other muscle branches such as to the sternocleidomastoid and anterior cervical muscles should be isolated as far into the muscle as possible. These muscle stumps were allowed to be uneven, which was inconsistent with the recommendations of Yamada et al [9]. The C_{3} nerve root and its branches were separated in the same manner. The distal end of C_{5,6} was also dissected to ensure the segments of the neural axis bundle were clearly visible. After the length of the grafts for the C_{3,4} nerve root was measured, the ipsilateral sural nerve trunk or sural nerve collateral branches were cut for transplantation. Except for phrenic nerve end-to-end anastomosis, there were still 4–5 bundles of the sural nerve needed between C_{4} and C_{5}, and another 4 bundles of sural nerve between C_{2} and C_{5}. The sural nerve transplanted to the broken end needed to be thick to avoid tension on the C_{5,6} root. Nerve anastomosis was performed using an operating microscope (Carl Zeiss, Zeiss Vario on S8, Germany; Figure 8). After the wound was sutured, patients were given postoperative antibiotics and neurotrophic drugs (Mecobalamin tablets, oral, 500 g, three times per day; Eisai, Tokyo, Japan). Patient muscle strength was evaluated, followed by electromyography.

**Figure 8** C_{3} and C_{4} + phrenic nerve transfer for treatment of C_{5,6} root avulsion.

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**Author contributions:** Lin Zou had full access to the study concept and design, performed animal experiments and statistical analysis, and drafted the manuscript. Xuecheng Cao was responsible for data analysis and paper validation. Jing Li performed the electrophysiology and provided data. Lifeng Liu was responsible for data analysis and integration. Pingshan Wang provided the funds for animal experiments. Jinfang Cai performed surgery and was in charge of the funding.

**Conflicts of interest:** None declared.

**Ethical approval:** This study was approved by the Medical Ethics Committee and Animal Ethics Committee of the General Hospital of Jinan Military Command of Chinese PLA.

**Supplementary information:** Supplementary data associated with this article can be found, in the online version, by visiting www.nrronline.org.

**REFERENCES**


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