NERVE TO MUSCLE NEUROTIZATION WITH SCHWANN CELL TRANSPLANTATION

+Fukuda, A; *Hirata, H; *Akeda, K; *Uchida, A
+Department of Orthopaedic Surgery, Mie University Faculty of Medicine, Japan

Introduction

Nerve to muscle neurotization (NMN) is reinervation of denervated muscles by direct implantation of transected nerve and has been used in experimental studies and clinical trials. However, NMN has seldom been recommended to use in clinical settings, because the technique can never yield useful muscle function. In contrast to denatured muscles by freeze-thawing or detergent which have been proven to be a potential substitutes for autologous nerve graft, fresh skeletal muscle is repulsive to regenerating axons. So, poor functional recovery after NMN may be the effect of the muscle tissue environment on axonal regeneration. It is generally recognized that Schwann cells play a key role in peripheral nerve regeneration. Recent reports have shown that intact peripheral nerves contain axonal growth inhibitory molecules such as myelin-associated glycoprotein (MAG) and chondroitin sulfate proteoglycans (CSPGs) which inhibit collateral sprouting and axonal extension, and thereby maintain nervous network. On nerve damage, dedifferentiated Schwann cells change distal nerve segment from repulsive to permissive environment not only by producing neurotrophic substances and neurokines but also by removing inhibitory molecules. In the present study, we investigated whether Schwann cell transplantation into denervated muscles can enhance functional recovery after NMN.

Materials and methods

All procedures were performed according to the protocols approved by the committee of animal research of Mie University. We used 36 adult male Lewis rats weighing about 300g. Primary cultures of Schwann cells were obtained from Wallerian degenerated adult rat sciatic nerves. Animals were anesthetized by inhalation of ether and then by intraperitoneal injection of hydrochloride. The left peroneal nerve innervating the anterior tibial muscle was resected. The tibial nerve was cut at the ankle level and the proximal stump was implanted into the denervated anterior tibial muscle under operating microscope. The animals were randomly divided into two groups. The denervated anterior tibial muscle was neurotized by tibial nerve implantation (group 1), and Schwann cell was transplanted in the neurotized muscle (group 2). Immunohistochemical study was carried out using anti-S100 antibody and anti-neurofilament antibody at 1,2,3,4,8,12 weeks after grafting. At 12 weeks after surgery, compound muscle action potentials and wet muscle weight were measured, and combined silver-acetylcholinesterase stain was used to identify reinnervated motor endplates (Fig. 1).

Results

Immunohistochemical study showed that regenerating axons and Schwann cells which grew into the muscle from the cut end of the transplanted nerve in group 2 were much more than group 1 (Fig. 2). The mean length of regenerating axons at one week in group 2 (628.4 μm) was greater than that in group 1 (207.4 μm). The compound muscle action potential and wet muscle weight in group 2 were significantly greater than those in the group 1. In group 1, the mean compound muscle action potential was 3.8mV and was 6.1mV in group 2. The study of muscle weight showed that the mean muscle mass was 62.3% of the left normal anterior tibial muscles in group 1 and was 79.7% in group 2. Combined silver-acetylcholinesterase stain clearly demonstrated that the number of the reinnervated motor endplates in group 2 (8.26/mm²) were much greater than in the group 1 (3.86/ mm²).

Discussion

The present study showed that Schwann cell transplantation was able to induce axonal sprouting and enhance reinervation of denervated muscles by NMN. The functional improvement by Schwann cell transplantation can be attributed to two independent events. Firstly, although little is known about the molecular mechanism of axonal growth inhibition in fresh skeletal muscle, accelerated axonal invasion seen in group 2 clearly demonstrated that transplanted Schwann cells changed repulsive environment for axonal growth in the muscle into permissive one. Secondly, significant increase in reinnervated motor endplates in group 2 strongly suggests that transplanted Schwann cells can contribute quantitative improvement in signal transmission at the neuromuscular junction. Recent reports showed that Schwann cells of the non-myelinating variety, called terminal Schwann cells, cover neuromuscular junction and participate in the maintenance and repair of these synapses. On muscle denervation and reinervation, activated terminal Schwann cells participate in inducing and guiding nerve sprouting at the neuromuscular junction. So, it is possible that transplanted Schwann cells functioned as activated terminal Schwann cells and enhanced neuromuscular reconnection. In conclusion, Schwann cell transplantation can be a useful adjunct to enhance reinervation of denervated muscles by NMN so as to recover muscle weight and useful strength.

Fig. 1. Combined silver-acetylcholinesterase stain (×100), showing motor endplates with reinervating axons.

Fig. 2. Immunohistochemical staining for s-100 and neurofilament, showing regenerating axons growing out from nerve stump at 4 week in group 1 (A), in group 2 (B).