SCIENTIFIC ARTICLE

Comparison of Neurotization Versus Nerve Repair in an Animal Model of Chronically Denervated Muscle

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Purpose Reinnervation of chronically denervated muscle is clinically unpredictable and poorly understood. Current operative strategies include either direct nerve repair, nerve grafting, nerve transfer, or neurotization. The goal of this study is to compare muscle recovery using microneural repair versus neurotization in a rat model of chronic denervation.

Methods Fifty-eight Sprague-Dawley rats had surgical denervation of the tibialis anterior muscle by transecting the common peroneal nerve. After 0, 8, 12, or 22 weeks of denervation, animals were assigned to either a direct repair or a neurotization cohort. An additional 7 animals were used for a sham cohort, and 7 of the 58 were used as controls. After a 12-week recovery period, animals had contractile strength and EMG testing of the tibialis anterior muscle. Peak force and characteristics were compared to the unoperated, contralateral limb. Tibialis anterior muscles were then harvested for mass and histologic evaluation.

Results Sixty-two animals completed testing. Denervated controls demonstrated a significant decrease in muscle mass, contractile strength, and peak motor nerve conduction amplitude compared to sham animals. In all groups, chronicity of denervation adversely affected functional recovery. On average, repair animals performed better than neurotization animals with respect to muscle mass, contractile strength, and peak motor amplitude. Differences in contractile force, however, were significant only at the 0 week denervation group (94% ± 30 vs 50% ± 20, repair vs neurotization). Neurotized muscles processed for histologic analysis demonstrated acetylcholinesterase activity at the nerve-muscle interface, confirming the formation of motor end plates *de novo*.

Conclusions We demonstrated that neurotization is capable of reinnervating *de novo* end plates in chronically denervated muscle. Our data do not support the hypothesis that direct muscle neurotization is superior to nerve repair for functional restoration of chronically denervated muscle. However, as the duration of denervation increases, the difference between outcomes of the neurotization and repair group narrows, suggesting that neurotization may offer a viable surgical alternative in the setting of prolonged denervation. *(J Hand Surg 2008;33A: 1093–1099. Copyright © 2008 by the American Society for Surgery of the Hand. All rights reserved.)*

Key words Chronic denervation, motor end plate, rat model, reinnervation.

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REINNERVATION OF CHRONICALLY denervated muscle is clinically unpredictable and poorly understood on a biological basis. Current operative strategies include either direct nerve repair, nerve grafting, or direct implantation of a peripheral nerve into a denervated muscle (neurotization), with the expectation that the nerve will grow and reinnervate denervated motor end plates.[1] Each of these strategies has been reported to result in partial clinical restoration of muscle function that correlates inversely with the duration of denervation. Some investigators have theorized that the principal site of failure of reinnervation is the motor end plate.[2,3] Others have demonstrated that Schwann cells within the distal nerve stump deteriorate and may be unable to regain the capacity for neuronal regeneration into muscle.[4] The goal of the current study was to compare the quality of muscle recovery in microneural repair versus neurotization in a rat model of chronic denervation.[4–6]

The specific aim of this study was to compare 2 surgical strategies for reinnervation of muscle after a period of chronic denervation by assessing the quality of muscle recovery using comparative mass, electrodiagnostics, histologic differences, and muscle strength recovery. The following 2 hypotheses are proposed:

Hypothesis 1: Cohorts treated with direct implantation of a peripheral nerve graft bridging a freshly cut donor motor nerve have the ability to reinnervate muscle and establish *de novo* motor end plates in chronically denervated muscle.

Hypothesis 2: Neurotization is superior to standard nerve repair for functional restoration of chronically denervated muscle.

MATERIALS AND METHODS

Surgical denervation and repair

The relative efficacy of direct nerve repair versus neurotization was examined using a rat model of chronic denervation. Experimental groups included cohorts treated by microneural repair (repair) and cohorts treated with neurotization (neurotization) via a peripheral nerve bridge after a period of 0, 8, 12, or 22 weeks of denervation.

An animal model described by Sulaiman and Gordon using Sprague-Dawley rats was used for experimental testing.[4] Animal surgery was performed under aseptic conditions and ketamine/xylazine anesthesia in accordance with an approved protocol from the Institutional Animal Care and Use Committee, Hospital for Special Surgery. Initially, 58 animals had surgical denervation of the right tibialis anterior muscle via a posterior incision. Sciatic, tibial, and peroneal nerves were exposed.[5,7] The right common peroneal (CP) nerve was identified, transected, and ligated with a 6-0 suture (Prolene, Ethicon, Inc., Sommerville, NJ) to prevent regeneration between the proximal and distal nerve stumps.[8] The distal ligated end was tacked to the popliteal fat for later identification. Wounds were then closed with nylon sutures and surgical staples. Animals recovered in an approved housing facility with monitoring of daily weights, postoperative Buprenex Hydrochloride (Reckitt Benckiser Pharmaceuticals, Richmond, VA) analgesia, and inspection of surgical sites.

After initial denervation, animals were randomly divided into 2 cohorts: 24 animals treated with microneural repair (repair) and 24 animals treated with neurotization (neurotization). Nerve reconstruction was performed immediately after transection (time point 0 weeks) and also after 8, 12, or 22 weeks of denervation. Six animals were allotted for nerve reconstruction for each time point (0, 8, 12, and 24 weeks) for each cohort. Thus at each time point, there were 6 animals having direct repair and 6 animals having neurotization.

In the repair group, the posterior incision was used to identify the sciatic nerve and the previously transected, tagged common peroneal stump. The motor branch of the tibial nerve was identified and confirmed with an 0.5 mA nerve locator stimulator (Bovie Medical Corporation, Melville, NY). Near its origin, the tibial nerve was transected and coapted to the freshly cut distal stump of the right CP nerve. Repair was performed with the assistance of a surgical microscope using simple 10-0 nylon sutures (Ethicon, Inc.) (Fig. 1A). A second sham anterolateral incision over the tibialis anterior was then made to ensure equal treatment to both neurotization and repair cohorts.

In the neurotization group, the posterior incision was again used to expose the sciatic nerve as it courses distally into the sensory and motor branches of the tibial nerve. The sensory branch of the tibial nerve was identified and ligated proximally at its origin and distally at the level of the ankle, then set aside for use as a reversed nerve graft. The sensory nerve was harvested because this reproduced a similar clinical scenario to humans in which a sensory nerve (eg, a sural nerve) might be harvested to use as an interposition graft. In addition, it limited morbidity to the contralateral leg for later comparison and it also minimized surgical time.

At its origin, the motor branch of the tibial nerve was transected and then coapted to the peripheral nerve graft using 2 simple 10-0 nylon sutures. After this was per-