SCIENTIFIC ARTICLE

Motor Nerve Recovery in a Rabbit Model: Description and Validation of a Noninvasive Ultrasound Technique

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Purpose To develop and validate a noninvasive ultrasound technique for the longitudinal analysis of functional recovery after segmental peroneal nerve reconstruction in a rabbit model.

Methods Twelve male New Zealand White rabbits underwent a 1-cm peroneal nerve autograft reconstruction. Ultrasound measurements were performed before surgery and at 1, 2, 4, 8, 12, and 16 weeks postoperatively. All rabbits were managed with manual restraint for the ultrasound procedure, avoiding the risks of anesthetics. At 12 and 16 weeks, we evaluated functional recovery using compound muscle action potential, isometric tetanic force measurements, wet muscle weight, and nerve histomorphometry. Data were compared with ultrasound measurements by calculating the Pearson correlation coefficient. We determined intra-rater and inter-rater reliability of the ultrasound measurements.

Results Ultrasound demonstrated good correlation with isometric tetanic force measurements and wet muscle weight, good correlation with nerve histomorphometry, and moderate correlation with compound muscle action potential. Both intra-rater and inter-rater reliability of the ultrasound technique was excellent.

Conclusions Ultrasound analysis of the tibialis anterior muscle provided a reliable method for analysis of functional recovery in a rabbit peroneal nerve reconstruction model. The noninvasive nature allowed for longitudinal follow-up within the same animal and measurement of early recovery without the use of anesthesia.

Clinical relevance Application of this noninvasive technique can reduce the variability and sample size necessary in peripheral nerve reconstruction studies and may provide an ideal tool for comparative studies in larger animal models. *(J Hand Surg Am. 2016;41(1):27–33.*

Key words Autograft, peripheral nerve injury, rabbit, tibial muscle, ultrasound.

The treatment of peripheral nerve injury represents a clinical challenge.[1] To study alternative treatment strategies for peripheral nerve repair, animal models are used.[2] Rats are frequently used and are especially useful as a first *in vivo step* to study short-term nerve regeneration because of their fast neuroregenerative capacity. However, important disadvantages impair the clinical relevance of such

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studies because of the rat's much faster nerve regeneration compared with humans and the limited length of nerve gaps that can be created in rats.[3] Those limitations can be overcome using rabbits, in which nerve gaps up to 8 cm are feasible.[4] Furthermore, the rabbit's neuroregenerative and immunological properties more closely mimic the human situation, providing an important step before clinical research.[5,6]

To evaluate motor nerve recovery after nerve reconstruction in both rat and rabbit, the easily obtainable wet muscle weight is frequently used. Other commonly used and established outcome measurements include isometric tetanic force measurement (ITF), a more accurate measurement of functional recovery, electrophysiological testing, and nerve histomorphometry.[7–9] However, all of those techniques require the animal to be killed for assessment, which restricts analysis of outcome over time. A commonly used noninvasive test is the sciatic function index. Unfortunately, this technique correlates poorly with other functional outcome measurements and is feasible only in the rat model.[10]

The use of ultrasound in a rat model has been shown to be both reliable and valid.[7,9] Allowing for multiple measurements over time within the same animal, ultrasound can reduce variability within the groups studied. This would ultimately reduce the required sample size and costs of these studies. We hypothesized that a similar technique could be used in the rabbit model as a noninvasive method for analyzing nerve reconstruction results. Therefore, the aim of this study was to investigate the reproducibility, reliability, and validity of a noninvasive ultrasound technique to measure the tibialis anterior muscle cross-sectional area to analyze functional recovery in a rabbit peroneal nerve injury model.

MATERIALS AND METHODS

After our institutional animal care and use committee granted approval, 12 male New Zealand White rabbits (Harlan Laboratories, Inc, Indianapolis, IN) (weighing 3–4 kg) underwent a 1-cm peroneal nerve autograft reconstruction on the left side. Animals were housed individually with a 12-hour light–dark cycle. Food and water were provided *ad libitum*.

Ultrasound measurements

The optimal ultrasound protocol was determined in a pilot study. Based on previous studies, we compared different probe positions to obtain the muscle cross-sectional area employing ultrasound using cadaveric rabbit legs (data not shown).[7,9,11] The protocol that both observers rated as most easily obtainable showed an SD of less than 5% for measurements within and between observers, and this protocol was chosen. All rabbits tolerated the ultrasound procedure with manual restraint in a Bunny Snuggle jacket (Lomir Biomedical, Inc, Malone, NY) and thereby avoided the risks of anesthetics. Ultrasound measurements were obtained before surgery and 1, 2, 4, 8 weeks after surgery and at the time of death, either 12 or 16 weeks after surgery. The hind limbs were shaved and remaining hair was removed using hair removal cream (Surgi-Prep; Miltex, York, PA). The experimental setup of the ultrasound measurements of the tibialis anterior muscle in the rabbit is depicted in Figure 1. Video 1 is an instructional video of the ultrasound protocol (available on the *Journal's* Web site at www.jhandsurg.org). With the ankle joint of the rabbit at 90°, the patella and lateral malleolus were identified. The ultrasound probe was placed perpendicular to the muscle one third of the way between those landmarks, and cross-sectional images of the muscle were obtained using the Philips CX50 ultrasound system with a 12- to 3-MHz linear transducer (Philips Healthcare, Eindhoven, The Netherlands) and ultrasound gel (Aquasonic 100; Parker Laboratories, Fairfield, NJ). Following the American College of Laboratory Animal Medicine and the Institutional Animal Care and Use Committee guidelines, animals were monitored for signs of discomfort.[12] At all time points multiple measurements were obtained by 2 similarly trained observers independently of each other. The cross-sectional area of the tibialis anterior muscle was analyzed in Adobe Photoshop CS5 Extended (Adobe Systems, Inc, San Jose, CA).

Surgical procedure

We induced anesthesia using 35 mg/kg ketamine (Ketaset; Fort Dodge Animal Health, Fort Dodge, IA) and 5 mg/kg xylazine (Vettek, Bluesprings, MO) administered intramuscularly. After a subcutaneous injection of 0.18 mg/kg buprenorphine (Buprinex; Reckitt Benckiser Pharmaceuticals, Inc, Richmond, VA), animals were intubated and maintained at spontaneous ventilation with the Bain ventilation system (Harvard Apparatus, Holliston, MA) under 1% to 2% isoflurane. Animals received intravenous lactated Ringer solution, and animal temperature was maintained at 37°C. All surgical procedures were performed under standard aseptic conditions. Through a longitudinal incision on the posterolateral aspect of the left thigh, the sciatic nerve and its peroneal branch were exposed by a muscle-splitting approach. Under a surgical microscope, a 1-cm segment of the peroneal