SCIENTIFIC ARTICLE

Validation of Isometric Tetanic Force as a Measure of Muscle Recovery After Nerve Injury in the Rabbit Biceps

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Purpose The purpose of this study was to describe and validate a technique for measurement of isometric tetanic force (ITF) in the rabbit biceps muscle.

Materials and methods Eighteen New Zealand White rabbits were randomized to test either the right side or the left side first. Under propofol anesthesia, the brachial plexus and biceps brachii were exposed. The middle trunk (C6, C7) was secured in a bipolar electrode. Compound muscle action potential (CMAP) was measured. The proximal, tendinous portion of the biceps was severed at the shoulder and clamped in a custom-made force transducer. Muscle preload and electrical stimulation variables were optimized to obtain the highest tetanic muscle contraction. Wet muscle weight (WMW) and nerve histomorphometry were analyzed. Statistical analysis was performed to determine side-to-side equivalence.

Results The rabbit biceps muscle force demonstrated side-to-side equivalence with overlapping 95% confidence intervals (95% CI). The right side, expressed as a percentage of the left, averaged 99.69% (95% CI, 88.89%−110.5%). The WMW of the right expressed as a percentage of the left was 98.9% (95% CI, 95.8%−102%).

Conclusions The ITF is equivalent from side to side in the rabbit as demonstrated by the high degree of overlap in the 95% CIs for each side. The width of the 95% CI implies that there is more variability in the rabbit upper extremity than for the lower extremity of the rabbit or rat models, and researchers should take this into account when performing sample size estimates in pre-experimental planning.

Clinical relevance The rabbit biceps muscle ITF measurements can be used to measure motor recovery in a rabbit model of brachial plexus injury and compared with the contralateral uninjured side. *(J Hand Surg Am. 2017;■(■):1.e1-e8.*

Key words Brachial plexus, motor function, rabbit model, peripheral nerve.

Online Exclusive

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Received for publication March 13, 2017; accepted in revised form October 31, 2017.

No benefits in any form have been received or will be received related directly or indirectly to the subject of this article.

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0363-5023/17/■ ■-0001$36.00/0
https://doi.org/10.1016/j.jhsa.2017.10.037

BRACHIAL PLEXUS INJURY IS ESTIMATED to occur in around 1% of trauma victims, with motorcycle and snowmobile accident victims at highest risk.[1] The overall incidence continues to rise as high-speed motor vehicle use increases.[2–5] Treatment of these injuries is challenging, outcomes can be unpredictable, and the best treatment for each injury remains controversial. Animal models of brachial plexus injury have primarily been limited to the rat

and mouse,[6–10] although the rabbit is gaining interest the literature.[11–16] Several authors have published observational anatomical studies,[14–16] and others have looked at root avulsion,[11] and nerve transfer in the rabbit biceps.[12,13] Despite this, no validated motor outcome measure has been described in the literature.

One successful strategy for evaluating peripheral motor nerve regeneration has been to use the maximum output force of the muscle innervated by the nerve in question. Comparison between injured and uninjured limbs is a practical means to determine total recovery and eliminate differences between specimens, which can reduce the number of animals needed.[17] Previously, validated studies of side-to-side equivalence of isometric tetanic force (ITF) in the rat tibialis anterior (TA) and rabbit TA muscles have been published.[17,18] In the rat, a side-to-side variability of 3.1% was established when 4 stimulation parameters were optimized. Although the rat sciatic nerve and TA muscle are useful in initial investigations of grafting and conduit techniques, rats appear to have superior regeneration capacity compared with humans. The size of the nerve gap is also limited in rats. The rabbit TA model was found to have a higher variability of 4.2% side to side when normalized for muscle weight.[18] The rabbit TA has the advantage of accommodating a longer nerve gap of up to 8 cm, and rabbits do not demonstrate the same enhanced neuroregeneration as seen in rats.[19]

To better model the complexity found in human brachial plexus surgery, a brachial plexus injury model using the middle trunk (C6, C7) and biceps muscle of the rabbit is described. The purpose of this study was to describe the technique for ITF measurement in the rabbit biceps muscle and to validate the side-to-side equivalence in an uninjured animal.

MATERIALS AND METHODS

After Institutional Animal Care and Use Committee approval, 18 male New Zealand White Rabbits weighing an average of 3.37 kg ± 0.04 kg underwent a nonsurvival experiment in which ITF of the biceps brachii was measured bilaterally. To minimize variability, right and left sides were randomized for initial testing. Compound muscle action potential (CMAP), wet muscle weight (WMW), length and width of the middle trunk, and nerve histomorphometry were also collected and analyzed.

Anatomy

Pilot anatomical dissections demonstrated that the rabbit biceps has only 1 muscle belly, homologous to the human long head of the biceps, which originates from within the shoulder joint at the glenoid. It receives innervation primarily from C7, with some contribution from C6. The location of nerve stimulation is the cranial portion of the caudal trunk.[14,15] This location is analogous to the human middle trunk, and we therefore refer to this as the middle trunk (Fig. 1).

Surgical procedure

Anesthesia was induced with an intramuscular injection of 35 mg/kg of ketamine (Ketaset; Fort Dodge Animal Health, Fort Dodge, IA), 5 mg/kg of xylazine (Vettek, Bluesprings, MO) and a subcutaneous injection of buprenorphine 0.18 mg/kg (Buprinex; Reckitt Benckiser Pharmaceuticals, Inc, Richmond, VA). Animals were intubated and kept on mechanical ventilation (large animal volume-controlled ventilator; Harvard Apparatus Company, Holliston, MA) with oxygen delivery at 1 L/min, tidal volume of 9 mL, and frequency of 25 breaths per minute. Propofol (Diprivan; AstraZenica, London, UK) 1% was infused intravenously at 0.6 to 1.5 mL/min to maintain anesthesia, adjusted based on corneal reflex. Two hundred milliliters of 5% dextrose solution was infused intravenously throughout the procedure. Temperature was measured by a rectal thermometer and maintained with a heating pad set at 37° C.

A 3- to 4-cm skin incision was made centered over the clavicle starting at the sternum and proceeding toward the shoulder. The subcutaneous tissue and muscle were divided to expose the middle trunk. The middle trunk was identified by its location just cranial to the subclavian artery. A 6- to 8-cm-long incision was made on the anterior upper limb from shoulder to elbow. The anterior deltoid muscle was separated from the middle deltoid in line with the humerus using bipolar cautery. The pectoral muscles were then detached from their insertion on the humerus from distal to proximal with care taken to avoid damaging the biceps muscle.

Compound muscle action potential: A miniature bipolar electrode (Harvard Apparatus Company) was applied to the middle trunk just after the union of C6 and C7 nerve roots. Two bipolar recording electrodes were placed into the biceps muscle and a ground electrode placed into the surrounding musculature. Using a VikingQuest Portable electromyograph and software (Nicolet Biomedical, Madison, WI), the CMAP was measured bilaterally using a stimulation duration of 0.02 ms and intensity of 2.3 mA. The position of the