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● *Original Contribution*

QUANTITATIVE EVALUATION OF DENERVATED MUSCLE ATROPHY WITH SHEAR WAVE ULTRASOUND ELASTOGRAPHY AND A COMPARISON WITH THE HISTOPATHOLOGIC PARAMETERS IN AN ANIMAL MODEL

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Abstract—This study explored the efficacy of shear wave ultrasound elastography (SWUE) for quantitative evaluation of denervated muscle atrophy in a rabbit model. The elastic modulus of the triceps surae muscle was measured with SWUE and compared with histopathologic parameters at baseline and at various post-denervation times (2, 4 and 8 wk) with 10 animals in each group. Our results revealed that the elastic modulus of denervated muscle was significantly lower at 2 wk but higher at 8 wk compared with that at the baseline ($p < 0.05$), and no significant difference was found between the elastic modulus at 4 wk and that at the baseline ($p > 0.05$). The wet-weight ratio and the muscle fiber cross-sectional area of the denervated muscle decreased gradually during the 8 wk post-denervation together with a gradual increase of the collagen fiber area ($p < 0.05$). In conclusion, SWUE was useful for quantitative evaluation of muscle denervation. The decreased elastic modulus might be an early sign of denervated muscle atrophy. (E-mail: wangyuexiang1999@sina.com) © 2017 Published by Elsevier Inc. on behalf of World Federation for Ultrasound in Medicine & Biology.

Key Words: **Muscle denervation, Shear wave elastography, Elastic modulus, Ultrasound.**

INTRODUCTION

Peripheral nerve injury is common. After nerve injury, the denervated muscle undergoes progressive atrophy that may lead to a loss of independence in activities, a higher risk of hospitalization and a reduced quality of life. Many procedures have been undertaken to reverse the process of muscle atrophy or to restore the function of the denervated muscle, such as electrical stimulation and injection of stem cells or muscle satellite cells, which have shown promising results (Chen et al. 2015; Pieber et al. 2015; Schaakxs et al. 2013; Shen et al. 2016; Su et al. 2016). However, some of these methods may be less effective if the targeted muscle has undergone irreversible atrophy. Thus, having the ability to evaluate the status of the denervated muscle at an early stage is clinically significant.

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Ultrasound has been used widely to detect muscle atrophy by evaluating the muscle quantity and quality. The thickness, muscle volume and muscle cross-sectional area (CSA) can be measured by gray-scale ultrasound and serve as useful parameters to track the changes in muscle architecture over time (Ikezoe et al. 2015; Mele et al. 2016; Mendes et al. 2015; Ng et al. 2015). The animal study by Mele et al. (2016) showed that a strong linear correlation was obtained between the ultrasonographic volume and the muscle CSA determined *ex vivo* on muscle cryosections. In addition, the study by Mendes et al. (2015) showed that, compared with controls, people with interstitial lung disease had a smaller CSA of the rectus femoris; moderate correlations were found between the rectus femoris CSA and knee extensor strength and elbow flexor strength. Yet, because of the variety of muscle size among healthy persons, it is difficult to establish a standard value for assessing muscle atrophy of a specific muscle. On the other hand, muscle size may not correlate well with muscle strength, which may decrease its significance in evaluating clinical patients (Baldwin and Bersten 2014).

Shear wave ultrasound elastography (SWUE) is a new imaging modality that evaluates the mechanical properties of the examined tissue. It uses acoustic radiation force to generate shear waves in soft tissue and subsequently reconstructs tissue viscoelasticity by tracking shear wave propagation speed (Garra 2007, 2011). Ultrasound elastography has recently been widely used to measure the elasticity of the liver, thyroid, breast and prostate and has shown its usefulness in differentiating malignant from benign lesions and staging of liver fibrosis (Correas et al. 2015; Lin et al. 2014; Liu et al. 2016; Phelps et al. 2017; Zeng et al. 2017). With regard to skeletal muscle, some studies have shown that assessing muscle stiffness was feasible and reliable with SWUE (Chernak et al. 2013; Koo et al. 2013; Nakamura et al. 2014; Shinohara et al. 2010). In patients with Parkinson's disease, the shear modulus of the affected muscles increased significantly compared with healthy muscles and correlated positively with the motion score (Du et al. 2016). In children with a cerebral palsy, the shear modulus of the lateral gastrocnemius muscle was significantly greater than that of unaffected children at all three foot positions: 20° plantar flexion, 10° plantar flexion and 0° plantar flexion (Brandenburg et al. 2016). However, few studies have been performed to evaluate the severity of denervated muscle atrophy with SWUE. Because of the pathologic changes of structure and substances in the denervated muscle, we hypothesized that the elastic modulus of the muscle might be different from the unaffected muscle and might gradually change after denervation.

The purpose of this study was to explore the elastic modulus changes of the denervated muscle on the basis of a sciatic nerve axotomy model in rabbits and to compare the elastic modulus results with the histopathological analysis.

MATERIALS AND METHODS

Animals

In this study, we used 40 healthy adult male Japanese White Rabbits (weighing 2.0–2.5 kg). The animals were obtained from the animal breeding center of Keyu, Beijing, China (license no. SCXK [Jing] 2012-0004). The experimental protocol was approved by the Ethics Committee of Chinese PLA General Hospital, Beijing, China (2015-x10-02). All experiments were performed in accordance with the Revised Guide for the Care and Use of Laboratory Animals. These animals were randomly allocated to four groups, using the computer software SPSS Statistics (v. 19.0, IBM, Armonk, NY, USA): group A (n = 10, 2.26 ± 0.14 kg, the control group without sciatic nerve axotomy at baseline of this study), group B (n = 10, 2.22 ± 0.12 kg, with sciatic nerve axotomy and 2 wk post-denervation), group C (n = 10, 2.23 ± 0.15 kg, with sciatic nerve axotomy and 4 wk post-denervation), group D (n = 10, 2.25 ± 0.16 kg, with sciatic nerve axotomy and 8 wk post-denervation). We used SPSS Statistics (v. 19.0, IBM) to determine the lower limb on which surgery would be performed (*i.e.,* the right or left side).

For the sciatic nerve axotomy, the rabbits were anesthetized intra-muscularly (IM) with 30 mg/kg pentobarbital sodium (Animal Drugs Ltd., Beijing Pubos Biotechnology Co., LTD, Beijing, China) and secured on the operating table. After routine sterilization, a 3-cm incision was made longitudinally at the posterior thigh 2 cm below the ischial tuberosity. The biceps femoris muscle was separated bluntly, and the sciatic nerve was exposed. The axotomy of the sciatic nerve was performed by excising a 1-cm length of the nerve tissue (Fig. 1). An antibiotic (penicillin 20,000 IU injected IM twice a day) was administered for 3 d post-operatively.

SWUE examination

The SWUE examination was performed with an Aixplorer ultrasound system (Supersonic Imagine, Aix-en-Provence, France) equipped with ShearWave Elastography. A linear array transducer SuperLinear L15-4 (4–15 MHz, [Supersonic Imagine]) was used to perform the ultrasound examinations. The SWUE technique is based on ultrafast ultrasound sequences that are performed to

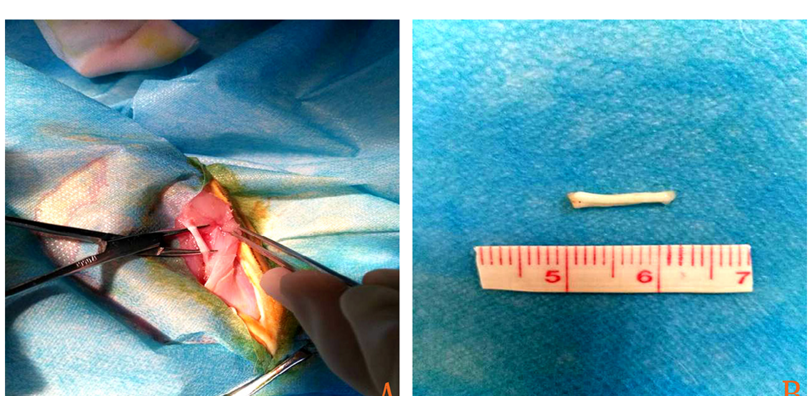

Fig. 1. (a) Exposing the sciatic nerve. (b) The sciatic nerve tissue was excised (1-cm length).