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Compensatory hypertrophy of the teres minor muscle after large rotator cuff tear model in adult male rat

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Background: Rotator cuff tear (RCT) is a common musculoskeletal disorder in the elderly. The large RCT is often irreparable due to the retraction and degeneration of the rotator cuff muscle. The integrity of the teres minor (TM) muscle is thought to affect postoperative functional recovery in some surgical treatments. Hypertrophy of the TM is found in some patients with large RCTs; however, the process underlying this hypertrophy is still unclear. The objective of this study was to determine if compensatory hypertrophy of the TM muscle occurs in a large RCT rat model.

Methods: Twelve Wistar rats underwent transection of the suprascapular nerve and the supraspinatus and infraspinatus tendons in the left shoulder. The rats were euthanized 4 weeks after the surgery, and the cuff muscles were collected and weighed. The cross-sectional area and the involvement of Akt/mammalian target of rapamycin (mTOR) signaling were examined in the remaining TM muscle.

Results: The weight and cross-sectional area of the TM muscle was higher in the operated-on side than in the control side. The phosphorylated Akt/Akt protein ratio was not significantly different between these sides. The phosphorylated-mTOR/mTOR protein ratio was significantly higher on the operated-on side.

Conclusion: Transection of the suprascapular nerve and the supraspinatus and infraspinatus tendons activates mTOR signaling in the TM muscle, which results in muscle hypertrophy. The Akt-signaling pathway may not be involved in this process. Nevertheless, activation of mTOR signaling in the TM muscle after RCT may be an effective therapeutic target of a large RCT.

Level of evidence: Basic Science Study, Physiology, Animal Model.

Keywords: Shoulder joint; rotator cuff tear; muscle hypertrophy; animal study; functional compensation

Rotator cuff tear (RCT) is a common musculoskeletal disorder in the elderly that results in the impairment of daily life due to intolerable shoulder pain or decreased muscle strength, or both. Previous epidemiologic studies have tried to reveal its prevalence and factors that may be

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involved in developing symptoms of RCT patients.[28,29] RCT occurs from within the rotator crescent tissues, which is the anterior part of the infraspinatus (ISP) tendon footprint, and then gradually extends posteriorly or sometimes anteriorly,[14,17,28] resulting in degeneration and atrophy of the torn rotator cuff muscles. In this condition, the integrity of the remaining TM muscle may affect some shoulder surgeries, such as reverse total shoulder arthroplasty or latissimus dorsi tendon transfer.[5,25] However, a human study reported that the remaining TM muscle becomes hypertrophic when the RCT extends to the ISP tendon,[13] and we have reported the possibility of compensatory hypertrophy of the ISP muscle in a RCT rat model.[11]

The Akt (protein kinase B) and mammalian target of rapamycin (mTOR) signaling pathways are important in the growth and hypertrophy of skeletal muscle. Akt acts as a critical signaling component for the regulation of various cellular functions.[3,7,8] In skeletal muscle, phosphorylation of Akt (p-Akt) activates protein synthesis and results in muscle hypertrophy.[15,19,23,24] mTOR is also known as a multiple regulator for cell size, proliferation, and survival and is considered a critical regulator for skeletal muscle hypertrophy. Phosphorylation of mTOR (p-mTOR) facilitates the phosphorylation of P70S6K and results in protein synthesis activation.[7,9,10,12,18,20,22] The inhibition of mTOR activity by rapamycin results in inhibition of skeletal muscle hypertrophy.[4] These reports indicate the possible involvement of the Akt- and mTOR-signaling pathways in hypertrophy of the TM muscle after RCT; however, such involvement has not yet been fully studied.

The objective of this study was to determine the compensatory hypertrophy of the TM muscle that occurs in our RCT rat model and whether Akt/mTOR signaling is activated during this compensation. We hypothesized that the detachment of the supraspinatus (SSP) and ISP tendons and transection of the suprascapular nerve (SSN) might alter the mechanical load in the TM muscle and activate the intracellular signal transduction pathway involved in the compensation.

Materials and methods

Animals

All efforts were made to minimize the number of animals used and the suffering of all animals. The study used 12 male Wistar rats (body weight, 240-260 g) that were purchased from SLC Japan (Hamamatsu, Japan).

SSN transection and SSP and ISP tendon detachment

The rats were divided into 2 groups for a biochemical experiment (n = 7) and a histologic experiment (n = 5). Under general anesthesia with ketamine (60 mg/kg body weight) and xylazine (12 mg/kg body weight, intraperitoneally), all rats underwent transection of the SSN and the SSP and ISP tendons of the left shoulder partially according to previous reports.[11,16,21] The right shoulder was left intact as a control. A 2-cm skin incision was made at the lateral aspect of the shoulder, and the underlying deltoid muscle was dissected bluntly along the muscle fiber to expose the rotator cuff tendons. A suture was passed under the acromion to apply upward traction for further procedures, and the trapezius muscle was dissected bluntly along the muscle fiber to expose the anterior aspect of the SSC muscle. The SSN was identified easily as it passes into the upper border of the SSC muscle vertically and was transected by surgical scissors.

The moment the SSN was transected, the left shoulder suddenly rotated externally. This sudden motion was considered to be a result of stimulation of the SSN and the subsequent contraction of the SSP and ISP muscles. The SSP and ISP tendon were identified and sharply detached at the insertion on the greater tuberosity using a scalpel blade. After the detachment, both tendons were partially removed, and the skin was sutured with 5-0 nylon. The rats were allowed unrestricted cage activity.

Sample collection and preparation

The rats were euthanized 4 weeks after surgery. We set this time point in accordance with our previous results that morphologic and molecular changes become evident within 4 weeks after surgery.[11]

The deltoid muscle was removed carefully without injuring the rotator cuff tendons. The SSP, ISP, and TM muscles of both forelimbs were removed from the scapula and humerus. The collected muscles were immediately weighed, frozen in liquid nitrogen, and stored at −80°C until used. Five samples were embedded with Tissue-Tek O.C.T. Compound (Sakura Finetek, Torrance, CA, USA) and frozen in cooled isopentane for histologic analysis. Seven samples were frozen directly in cooled isopentane for Western blot analysis. The muscle-to-body weight ratio was calculated by dividing the collected muscle weight by the total body weight (mg/g) and was used for the subsequent statistical analysis.

TM muscle cross-sectional area

The embedded TM muscle was sectioned with a thickness of 10 μm using a cryostat. Briefly, the lateral side of the muscle sample was directed to the front side, the ventral side was directed to the floor in the cryostat, and the muscle was sectioned at the middle body. This procedure was consistently performed in all samples, and the cranial, caudal, dorsal, and ventral sides of the sections were determined. The sections were stained with hematoxylin and eosin, and photomicrographs were obtained using the BZ-9000 system (KEYENCE, Osaka, Japan).

We divided the section into 4 regions: cranial-dorsal, cranial-ventral, caudal-dorsal, and caudal-ventral. We selected the same region in the control and operated-on sides and measured the cross-sectional area (CSA) of the muscle fiber with normal structures, which have no excessive space between the muscle fibers surrounded by an intact perimysium. The CSA of the muscle fiber was calculated using ImageJ software (National