J Shoulder Elbow Surg (2015) 24, 280-287

ELSEVIER

Journal of Shoulder and Elbow Surgery

www.elsevier.com/locate/ymse

Simvastatin reduces fibrosis and protects against muscle weakness after massive rotator cuff tear

Max E. Davis, BA[a], Michael A. Korn, BS[a], Jonathan P. Gumucio, BS[a,b], Julie A. Harning, BS[a], Anjali L. Saripalli, BS[a], Asheesh Bedi, MD[a], Christopher L. Mendias, PhD, ATC[a,b,*]

[a]*Department of Orthopaedic Surgery, University of Michigan, Ann Arbor, MI, USA*
[b]*Department of Molecular & Integrative Physiology, University of Michigan, Ann Arbor, MI, USA*

Background: Chronic rotator cuff tears are a common source of shoulder pain and disability, and patients with chronic cuff tears often have substantial weakness, fibrosis, inflammation, and fat accumulation. Identifying therapies to prevent the development of these pathologic processes will likely have a positive impact on clinical outcomes. Simvastatin is a drug with demonstrated anti-inflammatory and antifibrotic effects in many tissues but had not previously been studied in the context of rotator cuff tears. We hypothesized that after the induction of a massive supraspinatus tear, simvastatin would protect muscles from a loss of force production and fibrosis.

Methods: We measured changes in muscle fiber contractility, histology, and biochemical markers of fibrosis and fatty infiltration in rats that received a full-thickness supraspinatus tear and were treated with either carrier alone or simvastatin.

Results: Compared with vehicle-treated controls, simvastatin did not have an appreciable effect on muscle fiber size, but treatment did increase muscle fiber specific force by 20%. Simvastatin also reduced collagen accumulation by 50% but did not affect triglyceride content of muscles. Several favorable changes in the expression of genes and other markers of inflammation, fibrosis, and regeneration were also observed.

Conclusions: Simvastatin partially protected muscles from the weakness that occurs as a result of chronic rotator cuff tear. Fibrosis was also markedly reduced in simvastatin-treated animals. Whereas further studies are necessary, statin medication could potentially help improve outcomes for patients with rotator cuff tears.

Level of evidence: Basic Science, In Vivo Animal Study.

Keywords: Rotator cuff; fatty degeneration; muscle atrophy; statin; myosteatosis; fibrosis; HMG-CoA reductase inhibitor

Tears to the rotator cuff are among the most common and devastating upper extremity injuries, with more than a quarter of a million surgical repairs performed in the United States each year.[8] The ability to successfully repair the torn cuff and to promote the return of patients to normal strength and function is often complicated by fibrosis, atrophy, and fatty infiltration of the rotator cuff muscles.[5] These changes, termed myosteatosis or fatty degeneration, increase with time and are a

This work was supported by a research fellowship from the Alpha Omega Alpha Honor Medical Society, a research fellowship from Blue Cross Blue Shield of Michigan, and grants R01-AR063649 and T32-GM008322 from the National Institutes of Health.

This study was approved by the University of Michigan Institutional Animal Care and Use Committee (protocol 10500).

*Reprint requests: Christopher L. Mendias, PhD, ATC, Department of Orthopaedic Surgery, University of Michigan Medical School, 109 Zina Pitcher Place, BSRB 2017, Ann Arbor, MI 48109-2200, USA.

E-mail address: cmendias@umich.edu (C.L. Mendias).

1058-2746/$ - see front matter © 2015 Journal of Shoulder and Elbow Surgery Board of Trustees.
http://dx.doi.org/10.1016/j.jse.2014.06.048

limiting factor for adequate repair as well as for postoperative rehabilitation and recovery.[13,25] The extent of fatty degeneration can be quantified with magnetic resonance imaging or computed tomography techniques, and there is a positive correlation between the amount of fatty degeneration present in a muscle and poor functional outcomes as well as an increased risk for structural failure after repair.[15] Therapies that reverse or halt the progression of fatty degeneration may therefore lead to an improvement in function and greater patient satisfaction after rotator cuff tear.

Hydroxymethylglutaryl (HMG) coenzyme A (CoA) reductase inhibitors, or "statins," are among the most frequently prescribed medications in the United States.[31] These medications are most commonly used in the treatment of hypercholesterolemia as they are effective at lowering low-density lipoprotein cholesterol and improving clinical outcomes of patients with coronary artery disease and other cardiovascular conditions.[6,31] In addition to promoting cardiovascular disease, hypercholesterolemia is associated with a greater risk for rotator cuff tendon tear and impaired tendon-bone regeneration.[1,4] Aside from their efficacy in treating hypercholesterolemia, there are emerging roles for statins in the treatment of inflammatory diseases.[6,31] Statins work by inhibiting the activity of the HMG-CoA reductase enzyme, which catalyzes the conversion of HMG-CoA into mevalonate, a precursor for cholesterol and other isoprenoids that either directly or indirectly activate proinflammatory signaling pathways.[6] Numerous studies have identified the ability of statins to prevent fibrosis and inflammation in several diseased or injured tissues, including the heart, blood vessels, lungs, kidneys, skin, and articular cartilage.[2,6,23,32] To our knowledge, the ability of statins to prevent fibrosis, atrophy, inflammation, and fat accumulation in skeletal muscle tissue, and specifically the rotator cuff, has not been explored to date.

As therapeutic interventions to prevent muscle scar tissue formation and inflammation may enhance the treatment of chronic rotator cuff disease, our objective was to evaluate the ability of a commonly used statin medication, simvastatin (Zocor), to prevent atrophy and fibrosis after rotator cuff tear. We hypothesized that after an induction of a massive supraspinatus tear, simvastatin would enhance muscle fiber force production and prevent fibrosis and fat accumulation. To test this hypothesis, we used a well-described rat model of full-thickness chronic rotator cuff tear,[16,26,36] treated rats with either vehicle or simvastatin, and measured changes in muscle fiber type and contractility and biochemical and molecular markers of fibrosis and fatty degeneration 28 days after induction of tear.

Methods

Animals and surgical procedures

This study used 6-month-old male retired breeder Sprague-Dawley rats. Animals were housed in specific pathogen-free conditions and randomly assigned to either the control group (N = 8 rats) or the simvastatin treatment group (N = 8 rats). A bilateral full-thickness supraspinatus tenectomy was performed in each rat as previously described.[16,17] Rats were anesthetized with 2% isoflurane and placed in a lateral decubitus position, and the skin above the shoulder was shaved and scrubbed with chlorhexidine gluconate. A deltoid-splitting transacromial approach was used to visualize the supraspinatus tendon, which was then clamped and sharply detached from its insertion on the humerus. A full-thickness incision was made just distal to the myotendinous junction, and the tendon was removed to prevent healing and scarring into the surrounding connective tissue. A splash block of 1% lidocaine was administered for analgesia, and the deltoid was closed with 4-0 chromic gut (Johnson & Johnson, New Brunswick, NJ, USA). The skin was closed with a running subcutaneous suture of 5-0 Vicryl (Johnson & Johnson) that was reinforced with GLUture (Abbott Laboratories, Abbott Park, IL, USA). Rats also received subcutaneous buprenorphine (0.05 mg/kg) as analgesia postoperatively. After 28 days of recovery, the animals were anesthetized with sodium pentobarbital (50 mg/kg), and the supraspinatus muscles on both sides were harvested and weighed. The distal ends of all muscles were mobile and showed no sign of scar or lateral adhesions of the muscle. The rats were then humanely euthanized by overdose of sodium pentobarbital, which was followed by creation of a bilateral pneumothorax. The left supraspinatus from each rat was used for histology and single-fiber contractility, and the right supraspinatus was finely minced and used for gene expression and biochemical analysis.

Simvastatin administration

Pharmaceutical-grade simvastatin tablets (80-mg tablets; Cadila Pharmaceuticals, Ahmedabad, India) were finely ground with a mortar and pestle and extensively mixed with vehicle (1% hydroxypropyl methylcellulose) fresh daily. Rats received once-daily simvastatin at a dose of 20 mg/kg or vehicle (1% hydroxypropyl methylcellulose) administered by oral gavage. This dosage was selected on the basis of results from previous studies.[2,39] Treatment began 2 hours before the surgery to induce rotator cuff tear and continued each day until the rats were euthanized.

Muscle fiber contractility

The proximal portion of the left supraspinatus muscle was used for muscle fiber contractility analysis. Tissue was prepared, and the cross-sectional area (CSA), maximum isometric force (F_o), and specific force (sF_o, which is calculated by dividing F_o by CSA) were determined as described at a sarcomere length of 2.5 μm.[16,17,27] Ten to 12 type II fibers were tested from each supraspinatus muscle.

Histology

Histology was performed as previously described.[16,17] The distal portion of the left supraspinatus muscle was placed in Tissue-Tek (Sakura, Torrance, CA, USA) and frozen in isopentane cooled to approximately −160°C. Muscles were sectioned at a thickness of 10 μm and labeled with monoclonal antibodies against myosin heavy chain (Developmental Studies Hybridoma Bank, Iowa City, IA, USA). Primary antibodies were detected with Alexa Fluor conjugated secondary antibodies (Invitrogen, Grand Island, NY,