Evaluation of SPN-15 to Improve Mitochondrial Function in Rotator Cuff Tendinopathy

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INTRODUCTION: Tendinopathy results from cumulative injury due to repetitive overuse, leading to clinical symptoms of pain, reduced function, and decreased exercise tolerance. This pathophysiology poses a significant burden given the vital role of tendons in daily body movement. Tendinopathy has become a common condition observed in patients, with rotator cuff tendinopathy having an annual prevalence rate of up to 7.4%. Current therapies for tendinopathy generally do not address the underlying pathophysiologic changes, with no proven strategies to restore native tendon structure, composition, or mechanical properties of tendons. Mitochondria produce reactive oxygen species (ROS) during aerobic respiration and strain-mediated release of ROS. ROS overproduction can exacerbate mitochondrial dysfunction by damaging mitochondrial DNA, mitochondrial respiratory chain, and membrane permeability. Rotator cuff tendinopathy is associated with significant upregulation of ROS. Furthermore, prior studies demonstrated that mitochondrial dysfunction impacts the pathophysiology of degenerative tendinopathy. Mitochondria are an attractive target for drug therapy as they play a significant role in numerous diseases and aging. SPN-15, a 2nd generation mitochondria protectant, is a tetrapeptide attached to biocytin that targets and stabilizes cardiolipin on the inner mitochondrial membrane to enhance ATP production, reduce ROS production, and deliver biotin to mitochondria. This study aimed to evaluate the role of SPN-15 on supraspinatus tendinopathy using a murine subacromial impingement model.

METHODS: This study has been approved by The Weill Cornell Institutional Animal Care and Use Committee. Forty-two 12-week-old male C57BL/6J (Jackson Laboratory, Bar Harbor, ME) underwent a bilateral shoulder surgery where each experimental group consisted of 14 mice for a total of 28 limbs. The three groups were as follows: (I) control sham surgery, (II) 6 weeks of bilateral subacromial impingement, (III) 6 weeks of bilateral subacromial impingement with three weeks of intra-peritoneal SPN-15 treatment initiated three weeks after impingement surgery (5 mg/kg/d) (Fig. 1). All mice were sacrificed at six weeks following the initial impingement surgery. The supraspinatus tendons were harvested. Superoxide Dismutase (SOD) activity was measured with the SOD Activity Kit (Abcam) with absorption at 450nm. H&E, Alcian Blue, and Factor VIII IHC stains were performed for histology, and a modified Bonar Score was calculated by two blinded scorers. Biomechanical testing was performed using a custom MTS setup, loading specimens to failure at 1mm/min, measuring load-tofailure and stiffness. For gene expression analysis, cDNA was generated from total RNA (50ng) at 450nm. H&E, Alcian Blue, and Factor VIII IHC stains were performed for histology.

RESULTS: Biomechanical testing revealed a significant decrease in load-to-failure in the clip impingement (1.54N ± 0.46) and clip impingement + SPN-15 treatment (3.09N ± 2.06) groups compared to the sham control group (5.4N ± 1.47). The SPN-15 group demonstrated a slightly increased load to failure compared to the clip impingement group, although it did not reach significance (P=0.121). Similar results were seen for tendon stiffness (Fig 2A). SOD assay revealed that the sham surgery group and the clip impingement group did not have significantly different SOD activity, and the SPN-15 group demonstrated significantly increased SOD activity compared to the clip impingement group (p = 0.0398) (Fig 2B). Bonar scoring for histology revealed no difference between clip impingement and SPN-15 treatment groups for cell morphology, cellularity, ground substance, or vascularity. As expected, the sham tendon group exhibited significantly higher histology scores (more healthy appearing) than all impingement groups. TEM analysis revealed significantly decreased mitochondrial matrix density in the clip impingement group compared to the sham control that was restored with SPN-15 as matrix density significantly increased, and cristae structure was restored (Fig 3). The mitochondria in the clip impingement group showed reduced cristae density.

DISCUSSION: This study is the first to analyze SPN-15 in a tendinopathy model to understand its effects on mitochondrial dysfunction observed in tendinopathy. Biologic modulation using compounds such as SPN-15 may represent a viable option for the treatment of tendinopathy. We report from this study no gross histological or biomechanical differences between clip impingement controls and the SPN-15 treatment group. While load-to-failure and stiffness were improved with the administration of SPN-15, statistical significance was not met. However, SPN-15 may have utility in maintaining mitochondrial function as TEM demonstrated that mitochondria treated with SPN-15 appeared morphologically more normal. Increased SOD activity in cells treated with SPN-15 further supports the potential for a positive effect of SPN-15 in tendinopathy. While no gross structural differences were observed, SPN-15 may serve as an adjuvant to cells in surviving stressful micro-environments, such as in degenerative tendon. Along with other treatments, SPN-15 may serve as a therapy for tendinopathy. Limitations of this study include the lack of longer time points to further elucidate the treatment effect of SPN-15 and the differences in anatomy and function between a quadruped mouse and humans that cause this animal model to not perfectly replicate the pathophysiology of naturally occurring tendinopathy.

SIGNIFICANCE/CLINICAL RELEVANCE: Mitochondrial dysfunction has been identified as one pathway contributing to tendinopathy. SPN-15, a mitochondrial protectant compound, may serve as a potential therapeutic that ameliorates the mitochondrial dysfunction observed in tendinopathy.

IMAGES AND TABLES:

- Figure 1A - Study Design
- Figure 2A - Biomechanical Results of Supraspinatus Tendons. P < 0.05, ** P < 0.001, **** = P<0.0001
- Figure 2B - Superoxide dismutase activity
- Figure 3A-F - Representative TEM images of tendon cells. Fig. 3A.D Sham Control. Fig. 3B.E 6w Clip Impingement. Fig. 3F 6w Clip Impingement + 15mg/kg SPN-15 treatment. 3A-C 12000x, 3D-F 120000x
- Figure 3G - Mitochondrial density per group. * = P < 0.05, **** = P<0.0001