Multiple Corticosteroid Injections Compromise Tendon Histology and Biomechanical Properties in a Murine Rotator Cuff Tendinopathy Model

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INTRODUCTION: Rotator cuff tendinopathy secondary to subacromial impingement (SAI) is a common cause of shoulder pain[1]. Initial treatment typically involves a combination of physical therapy, oral anti-inflammatory medications, and local corticosteroid injections (CSI) which can be effective strategies for pain relief[2]. However, CSI use in rotator cuff tendinopathy remains controversial, as prolonged corticosteroid exposure can lead to decreased tenocyte viability, alterations in collagen composition, and decreased tendon mechanical properties[3-5]. Despite these known potential deleterious effects, CSIs are still commonly utilized in clinical practice, and there is no established consensus with regard to the timing and number of CSIs patients can receive. Previous pre-clinical studies have examined healthy and acutely transected rat rotator cuff tendons[4-5], but the consequences of CSI use have not been well-characterized in a murine rotator cuff tendinopathy model. The purpose of our study was to examine the structural, molecular, and functional effects of CSIs on tendinopathic murine supraspinatus tendons using a murine SAI model and to determine if multiple CSIs have a deleterious effect compared to a single CSI.

METHODS: The study protocol was approved by our institutional IACUC. Thirty 12-week-old male C57BL/6J mice underwent bilateral SAI surgery as previously described[6] and were randomized into two groups. One group received bilateral subacromial CSI (triamcinolone acetonide, 4uL) on post-operative day 3, followed by subacromial sham saline injections on post-operative days 7, 14, and 21. The other group received bilateral subacromial CSIs at all post-operative timepoints. All mice were sacrificed 42 days post-operatively for histological analysis, biomechanical testing, and bulk RNA-seq. Supraspinatus tendons were evaluated histologically for tendinopathic changes using a semi-quantitative modified Bonar scoring system by two blinded scorers. Biomechanical testing evaluated supraspinatus tendon maximum load (N), maximum stiffness (N/mm), maximum stress (MPa), and maximum modulus (MPa). RNA isolation was performed on supraspinatus tendon samples using a Qiagen Kit, and then bulk RNA-seq was performed. Gait analysis (DigiGait^{TM}) was performed at baseline (pre-operatively) and on post-operative days 3, 7, 14, 21, and 42. Statistical analyses were conducted with GraphPad Prism software using unpaired *t*-tests for histology and biomechanical testing, as well as two-way ANOVA with Tukey's post-hoc test for gait analysis. The significance threshold was set at $p < 0.05$ for histology, biomechanics, and gait analysis. RNA-seq significance was set at FDR < 0.05.

RESULTS: On histology, the multiple CSI group had greater ground substance deposition and higher vascularity within the supraspinatus tendon **(Fig. 1A,B)**. However, the total modified Bonar scores were not significantly different between the two groups. Tendons from the multiple CSI group were biomechanically weaker than the single CSI group, demonstrating significantly lower maximum force, maximum stiffness, and maximum modulus, although there was no difference in maximum stress **(Fig. 1C)**. There was no significant difference in the stance/swing ratio on gait analysis **(Fig. 2)**. The multiple CSI group had significantly longer stride length at 14 days and lower stride frequency on post-operative days 14 and 21 compared to the single CSI group **(Fig. 2)**, but these gait differences resolved by the 42-day timepoint. At 42 days, the multiple CSI group showed significantly higher expression of pathways related to inflammation and innate immune system function, cell-cell communication and cell metabolism, and nociceptive signaling **(Fig. 3)**.

DISCUSSION: The results of this study demonstrate that multiple CSIs in the setting of rotator cuff tendinopathy may provide some short-term functional benefits but can lead to worse tendon structural and mechanical properties. These findings add to pre-clinical evidence which suggests CSIs, especially multiple injections over time, can have deleterious effects on rotator cuff tendons. The multiple CSI group had significantly longer stride length and significantly lower stride frequency at intermediate timepoints, suggesting reduced pain with ambulation compared to the single injection group. However, there was no difference in stance/swing between groups at any timepoint, suggesting that any functional benefits associated with multiple CSIs were transient. Furthermore, the multiple CSI group had significantly higher expression of pathways related to inflammation, innate immune system function, and nociceptive signaling at 42 days. Our study highlights a key dilemma faced by physicians when treating rotator cuff tendinopathy: there is a trade-off between ameliorating pain and improving function in the short-term at the cost of potentially worsening long-term outcomes. A limitation of the study is the use of only male mice, although we plan to investigate sex as a biological variable in future studies with our model. Further investigation is necessary to determine optimal CSI dosing strategies, including the number of injections and timing relative to rotator cuff disease onset, to maximize symptom relief for patients without compromising tendon integrity.

SIGNIFICANCE/CLINICAL RELEVANCE: This study suggests that CSI use in rotator cuff tendinopathy may provide transient, short-term functional benefits but induces deleterious structural and molecular changes to tendon. Further research is needed to optimize injection strategies that alleviate symptoms without compromising tendon properties and ultimately long-term patient outcomes.

REFERENCES: 1) Harrison et al., *J Am Acad Orthop Surg*. 2011. 2) Horowitz et al., *Phys Med Rehabil Clin N Am*. 2023. 3) Puzzitiello et al., *Arthrosc Sports Med Rehabil*. 2020. 4) Chen et al., *J Steroid Biochem Mol Biol*. 2015. 5) Mikolyzk et al., *J Bone Joint Surg Am*. 2009. 6) Eliasberg et al., *J Orthop Res*. 2019. 7) Mohamadi et al., *Clin Orthop Relat Res*. 2017.

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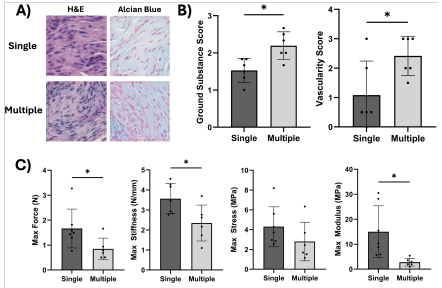

Fig 1. Histology and Biomechanical Analysis.
A) Representative histological images showing vascularity (H&E) and ground substance deposition (alcian blue) for both groups. Red boxes indicate 100 µm x 100 µm regions scored by histology graders. B) Modified Bonar scores for ground substance and vascularity demonstrated more extensive tendinopathic changes in the multiple CSI group. C) Biomechanical testing showed significantly lower max force, max stiffness, and max modulus in the multiple CSI group.

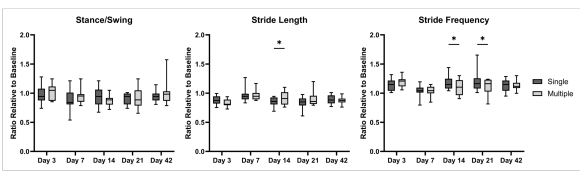

Fig 2. Gait Analysis.
There were no differences in stance/swing between the two groups at any timepoint. The multiple CSI group had longer stride length on day 14 and lower stride frequency on days 14 and 21.

Pathway	Database	logFC	FDR
DNA Mismatch Repair	WikiPathways	0.221	1.02E-04
IFNG Pathway	PID	0.222	1.20E-04
DNA Repair Pathways Full Network	WikiPathways	0.123	1.20E-04
Medicus Reference - Mismatch Repair	KEGG	0.218	3.92E-04
Processive Synthesis on the C Strand of the Telomere	Reactome	0.154	3.92E-04
SLC-Mediated Transmembrane Transport	Reactome	0.121	3.92E-04
Homologous Recombination	WikiPathways	0.155	4.04E-04
Neuroactive Ligand Receptor Interaction	KEGG	0.454	4.98E-04
Cell-Cell Communication	Reactome	0.250	5.63E-04
Brain-Derived Neurotrophic Factor Signaling	WikiPathways	0.059	5.63E-04
Fanconi Anemia Pathway	Reactome	0.083	8.50E-04
Initial Triggering of Complement	Reactome	0.598	1.14E-03
Cell Junction Organization	Reactome	0.226	1.18E-03
Nongenomic Actions of 1,25-Dihydroxyvitamin D3	WikiPathways	0.221	1.18E-03
Cardiac Progenitor Differentiation	WikiPathways	0.654	1.25E-03
Plasma Lipoprotein Assembly Remodeling & Clearance	Reactome	0.306	1.25E-03
Mitochondrial Oxidative Stress & Endothelial Dysfunction	WikiPathways	0.299	1.25E-03
Mismatch Repair	KEGG	0.210	1.25E-03
Homologous Recombination	KEGG	0.178	1.25E-03
Processing of DNA Double Strand Break Ends	Reactome	0.175	1.25E-03

Fig 3. Bulk RNA-seq Pathway Analysis.
The top 20 differentially expressed pathways in multiple CSI vs single CSI, as sorted by FDR. A positive log fold change indicates the pathway was more highly expressed in the multiple CSI group relative to the single CSI group.