Mast Cell Tryptase Modifies The Contraction Of Human Joint Capsule Cells In Vitro

Kevin A. Hildebrand, MD1, Lindsey M. Logan1, Mei Zhang1, David A. Hart, PhD1, Paul T. Salo, MD1, A. Dean Befus, PhD2.
1University of Calgary, Calgary, AB, Canada, 2University of Alberta, Edmonton, AB, Canada.


Introduction: Mast cells are an important component of inflammatory reactions, and tryptase is an abundant serine proteinase stored in mast cells. The presence of tryptase in blood or tissue fluids is an indicator of mast cell activation, and differentiates between mast cell-dependent and independent reactions. Mast cell tryptase acts as a growth factor for both fibroblasts and smooth muscle cells (1,2) and can cause proliferation of dermal (3) and cardiac (4) fibroblasts. Recent reports implicate a myofibroblast-mast-cell-neuropeptide axis of fibrosis in joint contracture formation. (5) Since tryptase is a major component of mast cell granules, it is hypothesized that tryptase plays a role in the development of joint contractures. To test this hypothesis, varying concentrations of β-tryptase were added to collagen gels containing human joint capsule cells (HJC) in the absence of mast cells, and the effects on gel contraction were measured.

Methods: Similar to previous experiments, (5) with prior approval from our ethics review board, anterior elbow joint capsules were obtained from 9 patients (3 men: 6 women; age 39±8yr, mean±SD) at the time of surgical release for chronic post-traumatic contractures (all > 1 year from injury). The capsules were minced, placed into T75 culture flasks and incubated at 37°C in a humidified atmosphere containing 5% CO2. The cells that grew in these cultures were passaged 4-10 times before being used in the study.

The HJC (2.5 x10^5 cells/mL) were mixed with collagen solution composed of 60% neutralized PureCol collagen I, 9.3% concentrated PBS (10x), 1.4% NaOH (1N), 9.3% FBS (10x), and 22% DMEM/F12 with 1x FBS and 1x antibiotic-antimycotic. Gelation occurred over 4-6h at 37°C in a humidified incubator containing 5% CO2. After gelation, the medium was changed to 500 µL serum free DMEM/F12 with 1x serum replacement and 1x antibiotic-antimycotic, with varying concentrations of human β-tryptase (10^-12, 10^-10, 10^-8, 10^-6, 10^-4 M; Sigma, St. Louis, MO) For the control medium, distilled water was added in place of β-tryptase. The gels were allowed to incubate for 1h before being released, and were photographed at 0h, 2h, 4h, 6h, 24h, 48h, and 72h post- release using ChemiDoc XRS. The contraction of gels was measured and the area of the gel calculated using Image J. The contraction was expressed as a percentage of the gel diameter at 0h. Statistical comparisons used a two way (treatment, time) ANOVA with a Posthoc Tukey test. Significance was set at p≤0.05.

Results: All collagen gels contracted over the first 24h, with significant differences between successive time periods in each group after 4h, or sooner for higher doses of tryptase. (Figure 1) The highest tryptase concentrations of 10^-6 and 10^-4, had the gel contraction stop after 48h and 24h, respectively, while at lower concentrations, contraction continued up to 72h. (Figure 1) After 4h all doses of tryptase except 10^-12 were significantly different from control for all times, except for the 48h and 72h control - 10^-4 group comparisons. There were some statistically significant differences between doses. The 10^-8
group was significantly different than the $10^{-12}$ group while the description of the other differences were complex due to the collagen gel contraction stopping in the $10^{-6}$ and $10^{-4}$ groups.

**Discussion:** The results indicate that tryptase can induce HJC to contract collagen gels at an increased rate when compared to HJC alone. The tryptase effect on HJC is concentration dependent, but the relationship is complex in this experimental model. In the concentrations tested, increasing levels led to greater contraction over the first 24 hours. However, with the highest doses ($10^{-6}$ and $10^{-4}$), contraction stopped after 48h and 24h, respectively. The lowest concentration tested did not differ significantly from the HJC alone. The results of this investigation indicate that the mast cell mediator tryptase may play a role in the development of post-traumatic joint contractures.

Given the effects of the higher and lower doses, it appears that the optimal concentration was included in the range tested. However, the reason for the concentration-dependent nature of the contraction is unclear. The lowest concentration tested followed the pattern of HJC alone and was not statistically different, establishing the lower dose range. However, the higher ranges had an initial contraction for at least 24 hours that then stopped in the experimental conditions defined. The cease in the contraction of the gels at concentrations of $10^{-6}$ and $10^{-4}$ suggests that tryptase may have modified the experimental conditions. It is known that tryptase, a serine protease, has the potential to degrade partially denatured collagen type I. (6) It may be that tryptase at higher concentrations is affecting the collagen gel itself.

Another possibility is a dose-dependent modification of the tryptase receptor. Tryptase acts on Protease Activated Receptor Type-2 (PAR-2). (3,4) PAR-2 is an inducible protein and its expression can be regulated by matrix composition. (7) By modifying the matrix, tryptase may have an indirect effect on the PAR-2 expression.

**Significance:** This research supports the hypothesis that mast cells are of central importance in the pathophysiology of joint contracture development. Future work is warranted to discover innovative therapies that target the mast cell, its mediators, and/or mediator receptors.

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A Prospective, Quantitative MRI-Based Assessment on the Progression of Fatty Infiltration after Rotator Cuff Repair

Drew Lansdown, MD, Sonia Lee, MD, Craig Sam, Roland Krug, PhD, Brian T. Feeley, MD, C Benjamin Ma, MD.
University of California, San Francisco, San Francisco, CA, USA.

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Introduction: Following tendon injury, the muscles of the rotator cuff undergo characteristic degenerative changes, including fatty infiltration and muscle atrophy. An increase in fatty infiltration has been shown to correlate with failure of surgical repair.(1,2) Rotator cuff repairs are performed frequently, with approximately 600,000 procedures per year, though there is continued debate about the role of surgical treatment in management of patients with rotator cuff tears.(3) To date, efforts on evaluating post-operative fatty infiltration have been limited by the clinical method for evaluating fatty infiltration, the Goutallier classification. This qualitative method has significant intra and inter-user variability, limiting the ability to detect small changes over time.(4) A quantitative MRI-based method using IDEAL fat measurements helps mitigate this by accurately determining the intramuscular fat fraction.(5) The purpose of this study is to evaluate the progression of fatty infiltration before and after surgical treatment using IDEAL MRI. We hypothesized that patients with small and medium tears would show lower progression compared to patients with large or massive tears.

Methods: A total of 10 patients (63.9 ± 10.1 years, 4 female) were recruited from a Sports Medicine and Shoulder Surgery practice at a tertiary, academic medical center. Patients provided informed consent, and all procedures were approved by our institutional review board. Patients underwent a baseline MRI prior to surgical treatment and a repeat scan 6 months after rotator cuff repair. MR imaging sequences included sagittal proton density fast-spin echo (FSE), sagittal T2-weighted FSE, coronal-oblique T1-weighted FSE, coronal-oblique T2-weighted FSE, and sagittal six-point IDEAL based on SPGR with T2* correction and 6 peak model (TR = 10.8 ms, BW = 62.5 kHz, ETL = 2, NEX = 1, FOV = 12 cm, alpha = 3, and resolution = 0.33 x 0.33 x 4.0 mm3). Image processing was performed with in-house, Matlab-based software. The supraspinatus (SS), infraspinatus (IS), subscapularis, and teres minor muscles were segmented. The first slice was chosen at the scapular-Y, along with one additional slice medial and two additional slices laterally. Using the IDEAL fat fraction map, the intramuscular fat fraction was calculated for each muscle. Images were reviewed by a fellowship-trained musculoskeletal radiologist to record pre-operative tear size, tendon retraction, post-operative repair integrity and post-operative tear size. Paired Student t-tests were used to compare the SS and IS fat fractions before and after rotator cuff repair. To investigate the effect of tear size, patients were divided into two groups: small/medium tears (tear size = 3 cm). Statistical significance was defined as p < 0.05.

Results: The mean pre-operative tear size was 2.34 cm. There were 6 patients in the small/medium tear group and 4 patients in the large/massive tear group. There was one repair that did not show evidence of healing in a patient with a combined SS/IS tear at baseline and evidence of SS healing but persistent IS tear at follow-up. The SS fat fraction was 8.6 ± 7.65% prior to repair and 9.45 ± 4.00% after repair (p =
For SS, the fat fraction was 6.64 ± 5.18% before repair and 8.19 ± 4.97% after repair (p = 0.38). The small/medium tear group showed significantly higher post-operative fat fractions (Figure 1). At baseline, the small/medium tear group SS fat fraction was 5.88 ± 2.24%, which increased to 8.91 ± 3.62% after surgery (p = 0.016). IS showed similar results, with baseline fat fraction of 4.41 ± 1.30% and follow-up fat fraction of 5.54 ± 1.32% (p = 0.012). The large/massive group had higher fat fractions at both time points, though there was not a significant difference between baseline and follow-up. For SS, the baseline fat fraction was 12.68 ± 11.42% and follow-up fat fraction was 10.27 ± 4.97% (p = 0.54). For IS, the baseline fat fraction was 10.00 ± 7.26% and after repair was 12.16 ± 6.01% (p = 0.66). The patient with healed SS but partial re-tear of IS demonstrated a 13% improvement in the SS fat fraction and an 11% worsening of the IS fat fraction.

**Discussion:** The preliminary results from this study show a small but significant increase in fat fraction of the SS and IS muscles following rotator cuff repair for small/medium tears. This relationship is not present in the group of patients with large/massive tears, where there was no significant difference in the fat fractions of SS and IS before and after surgery. Hamano et al. reported on 132 rotator cuff repairs and found that improvement in post-operative fatty infiltration was observed only in the large/massive tear group (6). This pilot study is part of an ongoing evaluation of pre and post-operative quantitative imaging of rotator cuff injuries and repair.

**Significance:** The results of this study suggest that fatty infiltration progresses following repair of small/medium tears though this change is not present for patients with larger tear sizes. Understanding the role that surgical repair of rotator cuff tears has on preservation of muscle quality will allow surgeons to better counsel patients on treatment options.

![Graph showing fat fractions](image)

**Figure 1.** Fat fractions of the supraspinatus and infraspinatus muscles at baseline and 6 months after rotator cuff repair. P values calculated through paired t-tests.

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