**Doxycycline-Mediated MMP Inhibition Improves Tendon-to-Bone Healing After Rotator Cuff Repair**

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**INTRODUCTION**

Matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes that have a critical role in tissue repair, degradation, and extracellular matrix homeostasis. Recent studies have demonstrated a potentially critical role of MMPs and tissue inhibitors of matrix metalloproteinases (TIMPs) in the pathophysiology of rotator cuff disease. Drugs which can modulate endogenous MMP activity to basal levels, and thereby reduce excessive tissue degradation or remodelling, may have a beneficial role in tendon-bone healing after surgical repair. The tetracycline family of antibiotics have been shown to inhibit mammalian collagenases by a mechanism that is independent of their antimicrobial activity, and recent studies have shown both local and systemic administration of tetracyclines to reduce bone and cartilage degradation associated with increased MMP activity. We hypothesize that (i) doxycycline-mediated MMP inhibition after rotator cuff repair will improve healing at the tendon-to-bone surface interface, and (ii) doxycycline-mediated MMP inhibition will variably affect tendon-bone healing when initiated at different temporal phases perioperatively.

**METHODS**

An established rat rotator cuff repair model was utilized and approved by the Institutional Animal Care and Use Committee at the Hospital for Special Surgery. 143 male, Sprague-Dawley rats underwent unilateral detachment of the right supraspinatus tendon from the greater tuberosity of the humerus followed by immediate repair using transosseous, non-absorbable suture fixation. Animals were divided into one control and three experimental groups. In the control group (n=46), the supraspinatus was repaired to its anatomical footprint. In the experimental groups, an identical surgery was performed with doxycycline hyclate (130mg/kg/day) administered in deionized drinking water at (i) preoperative day -1 (n=46), (ii) POD#5 (n=28), or (iii) POD #14 (n=23). Animals were sacrificed at POD#5, POD#8, 2 weeks, and 4 weeks postoperatively. The tendon-bone interface was assessed with quantitative histomorphometry for metachromasia and collagen fiber orientation as well as immunohistochemistry for collagen degradation products (COL2/3-short) (n=5/group). Assays for local MMP-13 activity at the tendon-bone interface using ELISA assay were performed at POD#8 and 4 weeks postoperatively (n=8/group). Stiffness and load-to-failure of the healing enthesis was tested at 4 weeks (n=10/group). Serum doxycycline levels were measured at the time of sacrifice in control and treated animals to measure the efficacy of drug delivery and correlate with measured MMP-13 activity at the enthesis (n=8/group). Statistical comparisons were performed using paired t-tests with significance set at p<0.05.

**RESULTS**

**Drug Delivery:** Doxycycline was effectively delivered to animals orally in their drinking water. Serum doxycycline levels at the time of sacrifice were significantly higher in all treated groups compared to control animals (1830±350ng/mL versus 3±3ng/mL respectively) (p<0.001).

**Histology:** The healing enthesis was cellular and demonstrated grossly similar morphology in the control and experimental groups. Doxycycline-treated animals demonstrated increased metachromasia at the healing enthesis at POD#5 (p=0.06), POD#8 (p=0.008), and 2 weeks postoperatively (p=0.04) (Figure 1). Evaluation of collagen birefringence revealed significantly improved organization in the doxycycline-treated groups compared to control animals at POD#5 (p=0.002), POD#8 (p=0.03), and 2 weeks (p=0.01) postoperatively (Figure 2). No significant difference in metachromasia or collagen birefringence was detected between any groups at 4 weeks postoperatively. Assessment of collagen degradation products at the tendon-bone interface using monoclonal COL2/3-short immunostaining as well as quantitation of local MMP enzymatic activity using ELISA assay is pending.

**Biomechanical Testing:** The healing enthesis of animals treated with doxycycline starting at preoperative day-1 or POD#5 demonstrated an increased load-to-failure compared to control animals (25.1±7.3N and 26.9±6.7N versus 19.6±4.4N respectively). However, these differences did not reach statistical significance (p=0.15).

**DISCUSSION**

Doxycycline-mediated blockade of MMPs in the perioperative period favorably influences healing at tendon-to-bone interface after rotator cuff repair. Administration of oral doxycycline at preoperative day-1 or POD#5 resulted in significantly increased fibrocartilage and improved collagen organization at the healing enthesis after rotator cuff repair. A trend towards increased biomechanical repair strength at 4 weeks in treated compared to control specimens was also observed. This may reflect the resilient and expeditious healing of the rotator cuff in a rodent model by 4 weeks postoperatively, and warrants testing at earlier postoperative intervals for detectable differences in repair strength.

Modulation of MMP activity after rotator cuff repair may offer a novel biological pathway to augment tendon-to-bone healing after rotator cuff repair.

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**Figure 1.** Doxycycline-treated specimens demonstrated increased metachromasia at the healing enthesis compared to controls at 5 days (p=0.06), 8 days (p=0.008), and 2 weeks postoperatively (p=0.04) (D@-1 – doxycycline started at preoperative day-1, D@+5 – doxycycline started at POD#5, D@+14 – doxycycline started at 2 weeks postoperatively).

**Figure 2.** Doxycycline-treated specimens demonstrated significantly improved collagen organization at the healing enthesis compared to controls at 5 days (p=0.002), 8 days (p=0.03), and 2 weeks postoperatively (p=0.01) as assessed by polarized light microscopy.