Doxycycline Improves Sedentary, but not Exercised, Supraspinatus Tendon and Muscle in a Rat Model

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Introduction

Matrix metalloproteinases (MMPs) have been implicated in the progression of tendon degeneration, but are also altered following non-injurious exercise. Potentially, one distinguishing feature between maladaptations and beneficial adaptations to exercise is the role of MMPs. MMP inhibition, through drugs like doxycycline, has been proposed to improve muscle and tendon healing.[1] Previous studies investigated doxycycline in acute injury models;[2-5] however, these studies were not designed to replicate chronic tissue adaptations. Therefore, the objective of this study was to investigate doxycycline on sedentary and exercised supraspinatus tendon and muscle using our rat model of non-injurious exercise.[6] With this model, we demonstrated distinct acute and chronic effects of exercise to the muscle and tendon and identified several altered genes associated with matrix turnover.[7] We hypothesized that doxycycline would abolish the beneficial adaptations present with exercise but have no effect on sedentary muscle and tendon properties.

Methods

One hundred seventy one male, Sprague-Dawley rats (400-450g, IACUC approved) were divided into 1 acute or 2 chronic time points and exercise (EX) or cage activity (CA) groups, as previously described.[6,7] Rats in the doxycycline (DOX) groups were administered an oil suspension of doxycycline hyclate (Wedgewood Pharmacy) orally (10mg/kg) every 24 hours.[3] Rats in the acute EX group were sacrificed 24 hours after a single bout of exercise (EX24). Rats in the acute DOX group began receiving DOX 24 hours prior to their exercise bout (EX24DOX). A separate group of rats maintained normal CA and received 3 doses of DOX until sacrifice 1.5 hours after their last dose (CA24DOX) and were compared to a non-drug treated CA group (CA24). For chronic time points, EX animals walked on a flat treadmill (10 m/min, 1 hr/day, 5 days/wk) for 2 or 8 weeks (EX2, EX8). Control animals maintained normal CA (CA2, CA8). DOX groups received DOX 7 days/wk for 2 or 8 weeks (EX2DOX, EX8DOX, CA2DOX, CA8DOX). Assays performed include supraspinatus tendon mechanical testing, tendon histology, and muscle histology. *Tendon Mechanics:* Mechanical testing protocol: 1) preconditioning, 2) stress-relaxation at 4% strain, 3) frequency sweep (0.1, 1, 2, 10 Hz) of 10 sine cycles, 0.125% strain amplitude, 4) return to gauge length, 5) stress-relaxation at 8% strain, 6) recovery to 4% strain, 7) return to 8% strain, 8) frequency sweep at 8% strain, 9) return to gauge length, 10) ramp to failure at 0.3%/s. Due to slip in the fixture that occurred near 4% strain, only the 8% data were analyzed (steps 5, 8, 10). *Tendon Histology:* 7μm paraffin sections of tendon were H&E stained and imaged with polarized light (chronic only) to determine the collagen alignment; cell density and shape were quantified with software (Bioquant). *Muscle Histology:* 10μm cryosections transverse to fibers were stained with anti-laminin and DAPI and analyzed[8] for centrally nucleated fibers and average fiber size. For fiber type analysis, sections were stained with anti-MyHC-I, MyHC-IIa, and MyHC-IIb and anti-laminin. Deep and superficial muscle regions[9] were analyzed for fiber type distribution and fiber type cross-sectional area. *Statistics:* To determine the effects of DOX, t-tests were used to compare DOX and non-drug treated groups separately for CA and EX at each time point. Significance: $p \leq 0.05$, Trends: $p \leq 0.1$. All data is presented as mean + standard deviation.

Results

Tendon Mechanics: Combined with a single bout of EX, DOX decreased tendon CSA and increased tendon modulus and max stress (Figure 1), bringing properties to within 0.2-10% of previously measured baseline levels. DOX also increased dynamic modulus (not shown). Viscoelastic parameters were not altered by DOX combined with acute EX. In chronic groups, DOX decreased tendon CSA and increased modulus for all groups (Figure 1) and increased dynamic modulus at all frequencies for the 2 week time point (EX and CA) and 8 weeks CA (not shown). DOX increased stiffness and max load in chronic CA groups but decreased stiffness and max load in chronic EX groups (Figure 1). Percent relaxation decreased with 2 weeks of CA but increased with 2 weeks of EX and 8 weeks of CA when combined with DOX (Figure 1). At 2 and 10 Hz, DOX reduced tan(δ) with 2 weeks of CA but increased tan(δ) with 2 and 8 weeks of

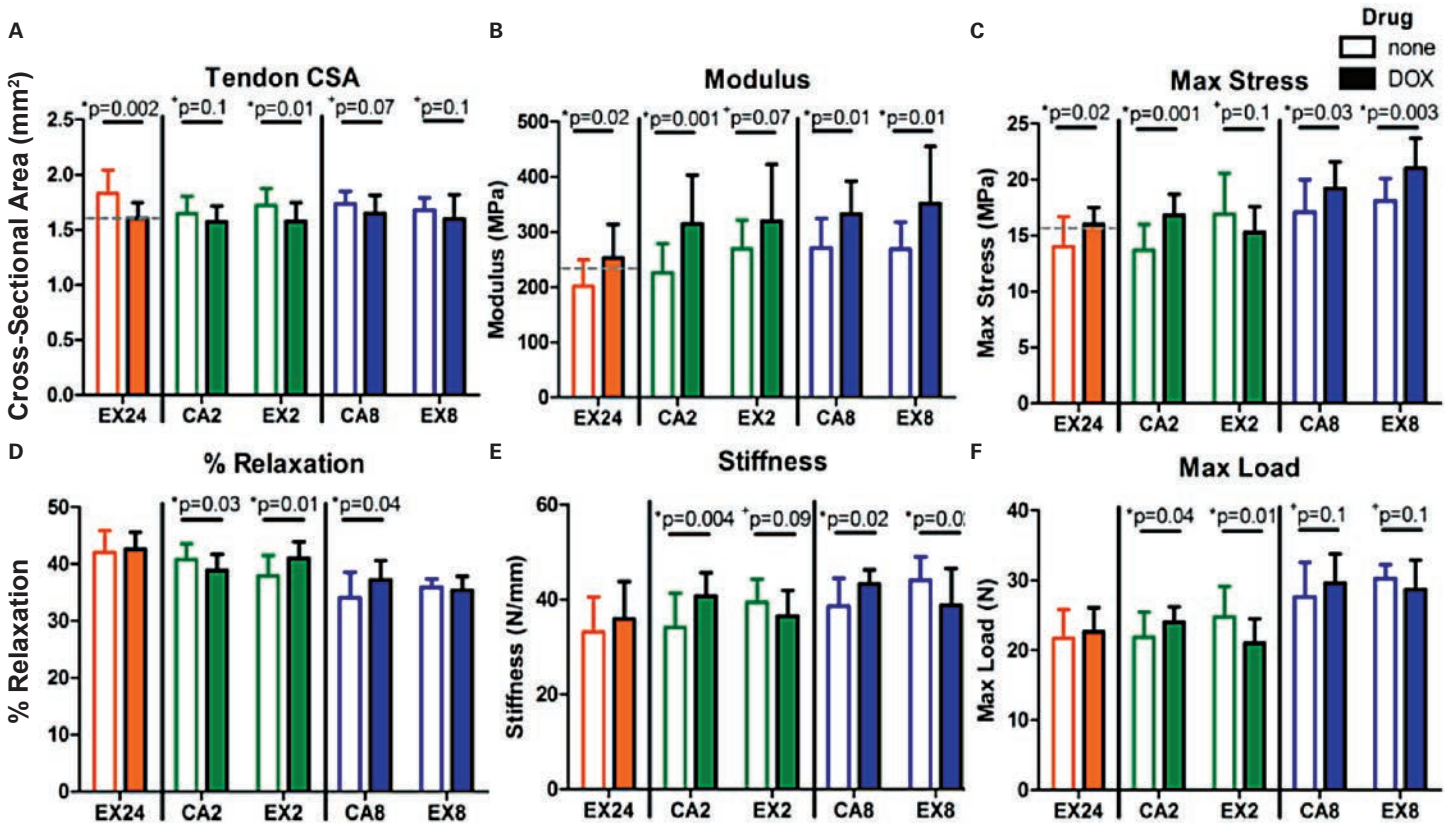

Figure 1. Tendon Mechanics. DOX combined with a single exercise bout brought tendon CSA, modulus, and max stress closer to baseline (gray dashes) by 24 hours. DOX reduced tendon CSA and increased modulus chronically. DOX had differential effects on % relaxation, stiffness, and max load in chronic CA and EX groups. n=10-15 acute, n=12-17 at 2 wks, n=10-14 at 8 wks.

EX (not shown). *Tendon Histology:* Acute administration of DOX had no effect on cell density or shape and only trended toward decreasing cell density when combined with a single bout of exercise (not shown). At 8 weeks, DOX decreased cell density with CA and EX. DOX resulted in rounder cells in the CA groups at 2 and 8 weeks and the EX group at 8 weeks (not shown). Tendon collagen organization increased with 8 weeks of DOX in the CA group (not shown). *Muscle Histology:* For all groups, the average percent of centrally nucleated fibers was below 1%, and DOX had no effect (not shown). DOX decreased the average muscle fiber CSA in all the EX groups but did not affect CA groups (Figure 2). Some muscle fiber type-specific changes were evident with DOX, including trends toward increased percent MyHC-IIa fibers, specific to CA groups (not shown).

Discussion

In contrast to our hypothesis, doxycycline significantly increased tendon mechanics and organization in sedentary groups. These increases were not always present with exercise. In fact, DOX combined with chronic exercise decreased stiffness and max load. We previously found MMP activity is higher in sedentary than exercised tendons, which helps explain these results. Our findings support previous studies that showed MMP inhibition of stress-deprived tendons prevented loss of mechanical properties.[10,11] When combined with exercise (but not CA), DOX decreased muscle fiber CSA, further suggesting that DOX combined with increased activity is not beneficial. In conclusion, results suggest that doxycycline at pharmaceutical doses induces beneficial supraspinatus tendon adaptations without negatively affecting the muscle in sedentary animals, supporting the use of doxycycline to combat degenerative processes; however,

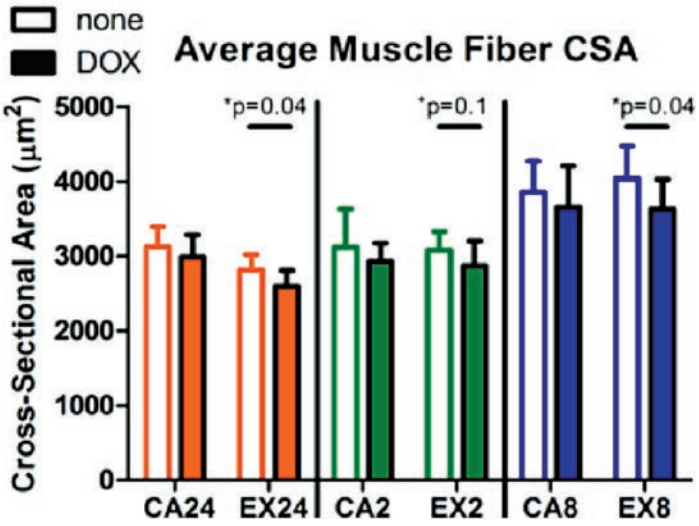

Figure 2. Muscle Fiber CSA. DOX decreased average muscle fiber CSA in EX, but not CA, groups. n=7-8 acute, n=7-9 at 2 wks, n=6-8 at 8 wks.

when combined with exercise, doxycycline does not produce the same beneficial adaptations in rat supraspinatus tendons and reduces muscle fiber cross-sectional area, suggesting that the drug is not advantageous when combined with activity.

Significance

Doxycycline, a commonly used drug, may be successful in preventing degeneration in tendon and muscle due to a sedentary state, but is not beneficial when combined with non-injurious exercise.

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