Static Tensile Strain Does Not Alter Tendon Response To Joint Inflammation In A Murine Explant Model

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Introduction: Secondary joint damage is thought to occur when inflammatory signals from one injured tissue interact with and cause injury in adjacent tissues within the joint. We previously showed in two different murine explant models that a low-dose dexamethasone treatment is beneficial in preventing the effects of secondary joint damage. However, our previous experiments were performed under stress-deprived conditions, which don't necessarily replicate the *in vivo* environment for secondary joint damage, in which tendons/ligaments would be uninjured and therefore continually subjected to tensile loads. The purpose of this study was to determine whether homeostatic mechanical loading would alter the response to injury-associated inflammatory signals.

Materials and Methods: Flexor digitorum longus (FDL) tendon explants were harvested from 60 male C57BL/6 mice at 4 months of age. Explants were subjected to either stress-deprivation ('SD') through free-floating culture or 3% whole tissue tensile static strain ('SS') using our custom-built tensioning device for a 7-day culture period. Explants were then treated with control medium ('Control'), injury-conditioned medium ('CM'), or 3-cytokine medium ('3C') with or without 100nM dexamethasone treatment ('Dex'). Control medium consisted of low glucose DMEM supplemented with 10% fetal bovine serum and 1% antibiotic solution. CM was collected from our previously established secondary damage injury model as described. To create 3C medium, 10 ng/ml IL-1β, 10 ng/ml IL-6, and 10 pg/ml TNF-α were added to control medium. All treatments were added at day 2 and replenished every two days. After 7 days, explants were assessed for viability through live/dead staining, metabolic activity through the resazurin reduction assay, and cell proliferation and glycosaminoglycan (GAG) synthesis through ^{3}H-thymidine and ^{35}S-sulfate radiolabeling, respectively. We also measured DNA, GAG, and collagen content through standard biochemical assays. One-way ANOVAs were performed followed by Bonferroni corrections with significance at $p<0.05$ (*).

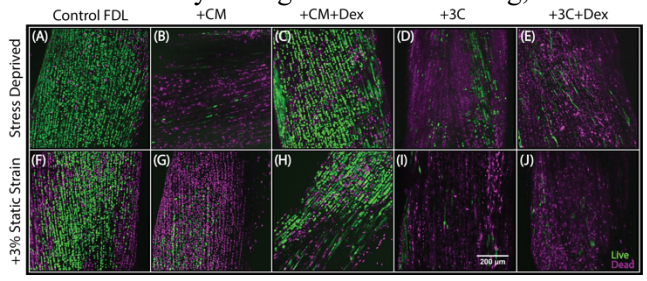

Figure 1. Day 7 live/dead viability for flexor explants in SD or SS conditions treated with (A,F) control medium,

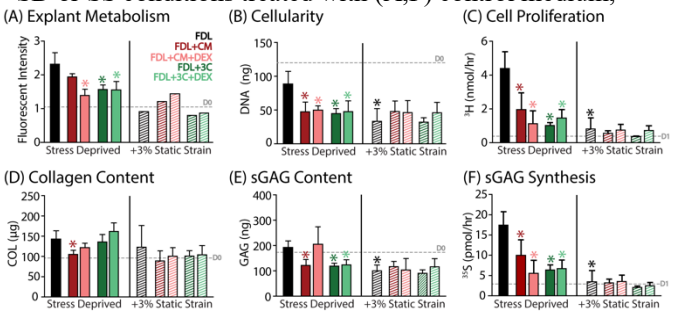

Figure 2. Day 7 Explant (A) metabolism, (B) cellularity, (C) cell proliferation, (D) collagen, (E) GAG, (F) GAG synthesis. * = significant vs. day 7 SD controls (solid black).

Results and Discussion: Viability (Fig. 1) was reduced in SD explants incubated in CM or 3C medium for 7 days. Treatment with Dex was able to prevent the loss of viability in CM explants, but not those subjected to 3C. This result was unchanged with SS, demonstrating that inflammation-induced cell death and the benefit of Dex are not mechanically sensitive. However, SS alone did alter control explants by reducing almost every outcome measure (Fig. 2). Values of metabolic activity, proliferation and GAG synthesis were similar to those measured early in the culture period (day 0 or 1 [grey dashed lines in Fig. 2]), suggesting that SS maintained cell activity closer to *in vivo* values. In response to CM or 3C incubation, SD explants had reduced matrix content and cell activity. Dex prevented this loss in CM explants, but not 3C explants. Interestingly, this result was not detected in SS conditions. There were no differences in composition with CM or 3C, suggesting that SS may be protective of matrix degeneration. There did appear to be decreases in cell proliferation and GAG synthesis with CM and 3C that were rescued by Dex, but due to large decreases in control values we were unable to detect statistical significance. Ongoing studies are investigating other loading scenarios, such as cyclic loading.

Conclusions: While static mechanical strain may be protective of matrix loss caused by secondary joint damage, it does not prevent inflammation-associated cell death. Furthermore, low-dose dexamethasone treatment does show significant benefit in preventing secondary damage-related tendon injury.

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