Low-Dose Dexamethasone Prevents Inflammation-Induced Cell Death and Injury-Like Response in Flexor Tendon-Only and Rotator Cuff Bone-Tendon-Muscle In Vitro Explant Models

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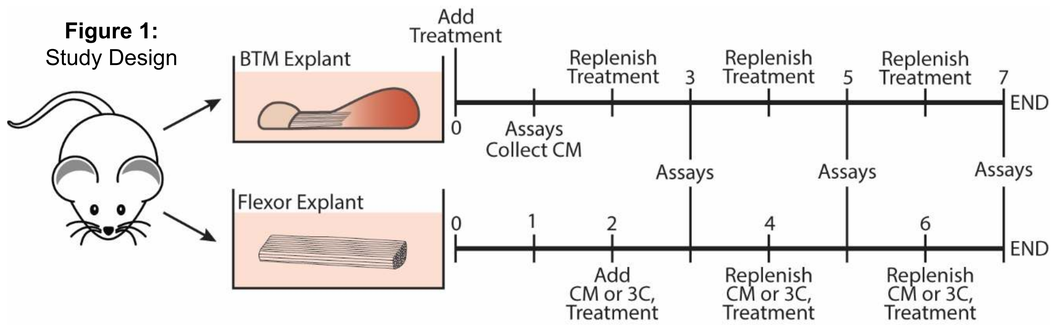
BACKGROUND

- Injured and uninjured joint tissues interact through biochemical and mechanical cues [1,2]
- We recently developed a novel rotator cuff explant culture model which contains muscle, tendon, and bone together for the first time [3]
  - Pro-inflammatory cytokines (IL-6, TNF-α) released from muscle and bone caused a loss of viability in the absence of a specific tendon injury
- **Purpose:** Investigate how pro-inflammatory cytokines influence tendon health in an explant model and explore potential treatments to prevent loss of viability and degeneration
- **Hypotheses**:
  1. Cytokines induce an injury-like response with reduced viability and matrix degeneration
  2. A broad-spectrum glucocorticoid will be more effective than single cytokine inhibitors at preventing the effects of inflammation-induced tendon injury

METHODS

- **Preparation:** Bone-tendon-muscle (BTM) explants [humeral head-supraspinatus tendon-muscle] and flexor digitorum longus (FDL) tendons from 4-month old C57BL/6J mice
- **Culture and Medium:** Explants cultured in stress-deprived conditions for up to 1 week
  - *Control:* Low glucose DMEM + 10% fetal bovine serum + 1% antibiotic solution
  - *CM*: 1:1 Mixture of BTM 24-hour explant medium mixed with Control medium
  - *3C*: Control medium + 10ng/mL IL-1β + 10ng/mL IL-6 + 10pg/mL TNF-α
- **Therapeutic Treatments:** 100nM Dexamethasone ('Dex'), 2.5 μg/mL etanercept ('EN'), or 100ng/mL IL-1RA ('RA'); All added to culture medium at the onset of inflammatory insult

Figure 1: Study Design

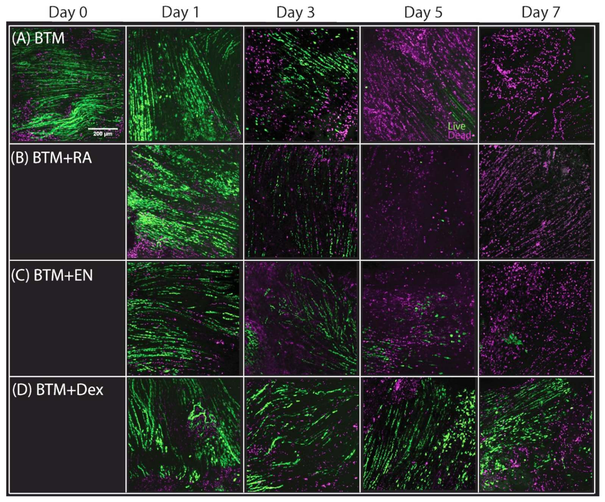


- **Cell Viability:** Live/dead confocal imaging via fluorescein diacetate/propidium iodide stain
- **Metabolism:** 3-hour resazurin → resorufin reduction assay
- **Biosynthesis:** 24-hr addition of ^{35}S-sulfate; **Cell Proliferation:** ^{3}H-thymidine
- **Composition:** Sulfated GAG content by DMMB assay, DNA content by PicoGreen assay, and total collagen by OHP assay (All performed on proteinase K digests of tissues)
- **Statistics:** 2-way ANOVAs with Bonferroni-corrected post-hoc tests. Data are presented as mean ± 95% confidence interval unless otherwise noted, with color-coded symbols to represent post-hoc comparisons

✱ Compared to Control FDL/BTM
¤ Compared to CM or 3C

RESULTS

Figure 2: Viability images of BTM explants treated with (A) control medium or medium supplemented with (B) IL-1RA, (C) etanercept or (D) dexamethasone.



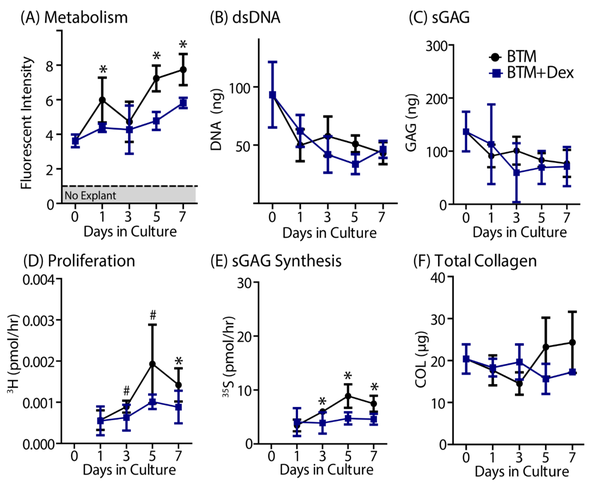


Figure 3: (A) Explant metabolism (bone+tendon+muscle) and tendon (B) DNA content, (C) GAG content, (D) cell proliferation, (E) GAG biosynthesis, and (F) collagen content for BTM control (black) and BTM+Dex (blue) groups.

RESULTS

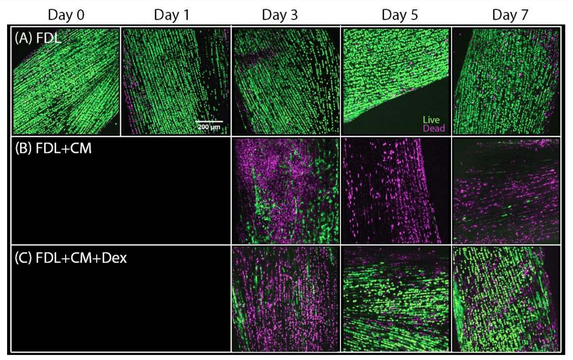


Figure 4 (above): Viability images of FDL explants in (A) control FDL, (B) FDL+CM or (D) FDL+CM+Dexamethasone.

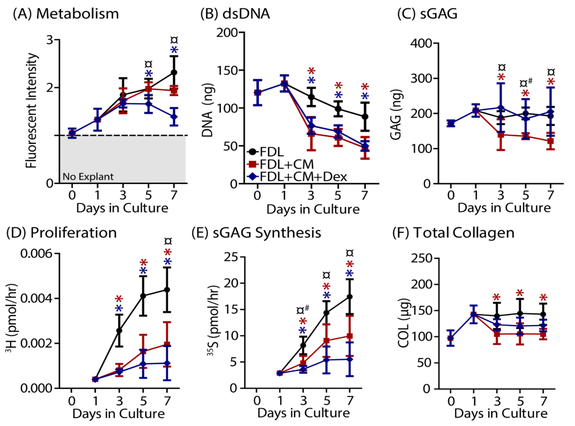


Figure 5: (A) Metabolism, (B) DNA content, (C) GAG content, (D) cell proliferation, (E) GAG synthesis, and (F) collagen content for FDL control (black), FDL+CM (red), and FDL+CM+Dex (blue) groups.

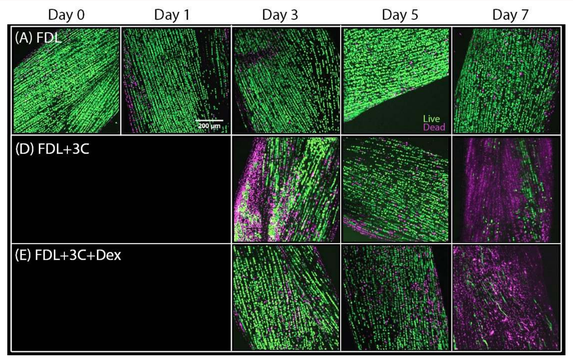


Figure 6 (above): Viability images of FDL explants in (A) control FDL, (B) FDL+3C or (D) FDL+3C+Dexamethasone.

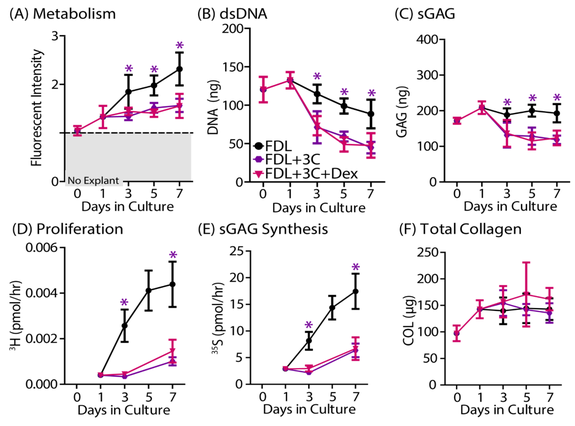


Figure 7: (A) Metabolism, (B) DNA content, (C) GAG content, (D) cell proliferation, (E) GAG synthesis, and (F) collagen content for FDL control (black), FDL+3C (violet), and FDL+3C+Dex (magenta) groups.

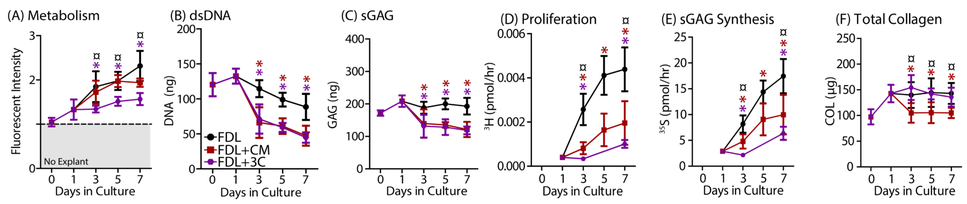


Figure 8: (A) Metabolism, (B) DNA content, (C) GAG content, (D) cell proliferation, (E) GAG synthesis, and (F) collagen content for FDL control (black), FDL+CM (red), and FDL+3C (violet) groups.

DISCUSSION

- Pro-inflammatory cytokines impact tenocytes in the absence of tendon injury (Fig. 4-8)
- Incubation of healthy FDL explants in CM and 3C medium causes a loss of viability and degeneration of the tendon matrix, confirming results in BTM explants
  - CM causes loss of viability in 24h while 3C takes up to 5 days (Fig. 4,6)
- Neither targeted cytokine treatment prevented loss of viability or degeneration of matrix in BTM (Fig. 2, other data not shown) or FDL explants (not shown)
- Low dose Dex prevents loss of viability in BTM and FDL+CM (Fig.2-5)
  - Reduced metabolism, proliferation and biosynthesis, bringing closer to *in vivo* levels
  - Suggests Dex can be protective of cell viability in explant culture, mirroring results in injured cartilage induced through *in vitro* mechanical and biochemical injury [4]
- Dex did not prevent or rescue degenerative changes causes by 3C medium
  - Conditioned medium (CM) is likely more than just pro-inflammatory cytokines (Fig. 8); Other factors may be critical to developing and testing therapeutics for joint injuries
- Monolayer studies suggest negative effects of Dex but effects appear to be dose- and time-dependent, and affected by presence of native ECM; future studies will investigate
- Future studies include influence of (1) loading/exercise, (2) aging, (3) sex

REFERENCES

[1] Haslauer+2013, Osteoarthritis Cartilage, 21(12):1950-7. [2] Reuther+2012, J Orthop Res, 30(9):1435-9. [3] Connizzo+2018, Connect Tissue Res, Jun 6:1-14. [4] Grodzinsky+2017, J Orthop Res, 35(3):406-11.

ACKNOWLEDGEMENTS

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