Triamcinolone Acetonide Has Minimal Effect on Short- and Long-Term Metabolic Activities of Cartilage

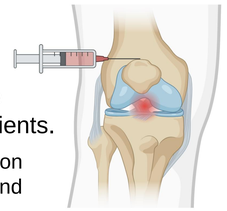
Annie Porter, Emily Newcomb, Steven DiStefano OMS-3, Michael Axe, X. Lucas Lu

University of Delaware, Newark, DE USA

Introduction

Corticosteroids are commonly used to **manage joint inflammation.**

- **Triamcinolone acetonide (TA)** is the most prescribed corticosteroid for intra-articular knee injections [1].



Conflicting studies on whether TA damages cartilage has caused **clinicians to fear** using TA in young patients.

- Influence of TA on cartilage remains unclear depending on methods of *in vitro* cell culture, *in vivo* damage model, and dose of TA [2].

Aim: Evaluate the effects of Triamcinolone Acetonide on *in situ* chondrocyte in young healthy cartilage

Studied **physiologically relevant doses** of TA on chondrocytes in their native matrix.

- *Maximum TA dose*: highest possible solubility of TA for 14 days continuously.
- *Minimum TA dose*: sustained concentration in joint from slow-release particles (Zilretta®).

Methods

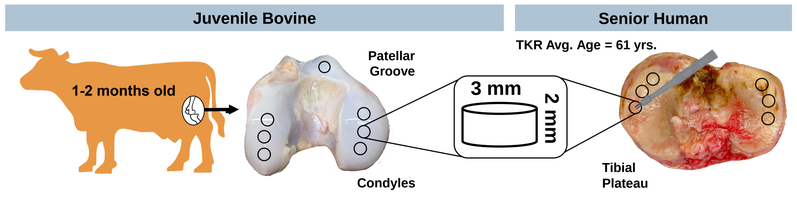


Fig. 1 Cartilage tissue harvest. Juvenile bovine cartilage explants were harvested from the knee joints of calves. Senior human cartilage explants were harvested from the tibial plateaus of surgical waste (5F/4M). Samples were cultured in chondrogenic media for 48 hrs before any experiments.

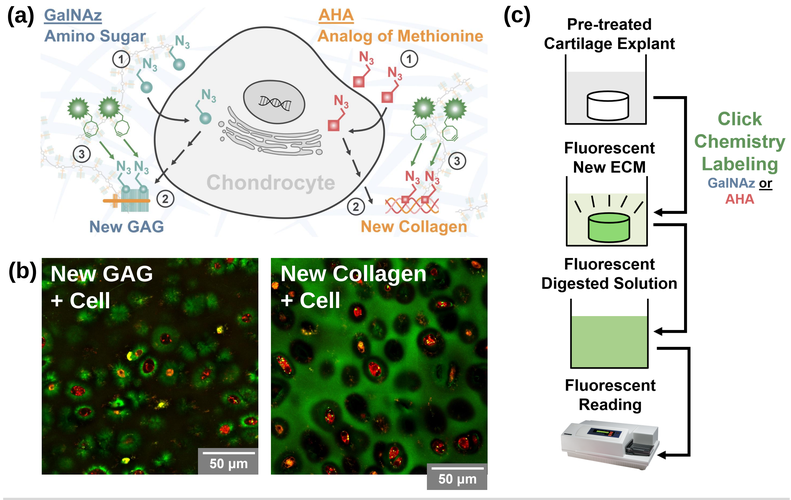
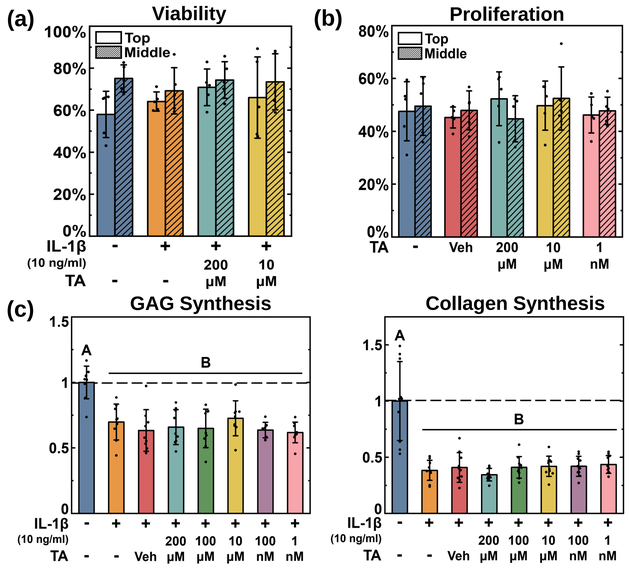


Fig. 2 Click chemistry method to quantify ECM synthesis and degradation. (a) Azide-labeled biomolecules (GAG or collagen) are tagged with a fluorescent dye via a bioorthogonal click chemistry reaction [3]. (b) Newly synthesized GAG or collagen (green) surrounding chondrocytes in cartilage (red). (c) Quantification of new GAG or collagen synthesis labeled using the click chemistry method.

Results

Juvenile Bovine Cartilage



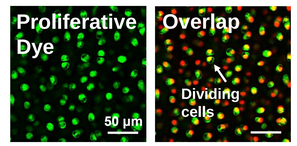


Fig. 3 Short-term effects of TA. Cartilage treated with TA for 48 hrs with or without inflammation (IL-1β; 10 ng/ml). TA had no effect on chondrocyte (a) viability or (b) proliferation. Chondrocyte proliferation evaluated with click chemistry method. IL-1β reduced (c) GAG and collagen synthesis, which TA did not improve but also did not worsen. Different letters indicate significant differences between groups (p<0.05).

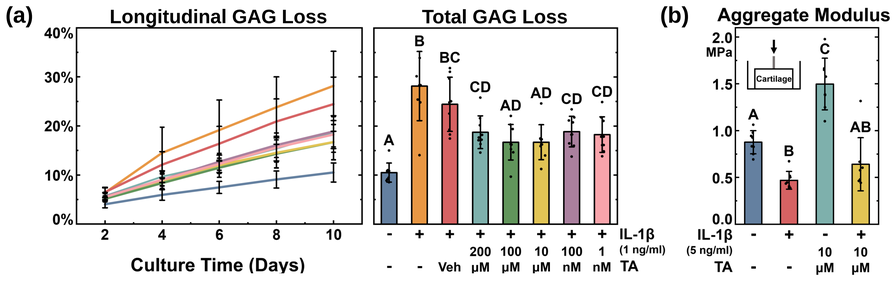


Fig. 4 Long-term effects of TA on cartilage degradation. (a) Longitudinal and total loss of GAG from cartilage during a continuous 10-day IL-1β (1 ng/ml) exposure and TA treatment. IL-1β induced ~30% loss of GAG, which was reduced to <20% by all doses of TA. (b) Cartilage was exposed to 5 ng/ml IL-1β for 10 days with or without simultaneous TA treatment (10 μM). Mechanical properties were evaluated with micro-indentation testing and biphasic curve fitting. TA rescued the aggregate modulus which had been reduced by inflammation. Different letters indicate significant differences between groups (p<0.05).

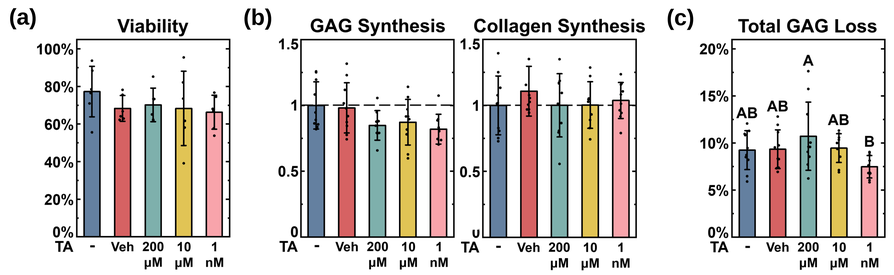


Fig. 5 Long-term effects of TA alone. Cartilage treated with TA alone for 14 days. TA had no significant effects on (a) chondrocyte viability or (b) GAG and collagen synthesis rates. (c) Treatment with TA did not induce any significant GAG loss compared to control. A 1 nM dose of TA did reduce the amount of GAG loss compared to a 200 μM dose. Different letters indicate significant differences between groups (p<0.05).

Senior Human Cartilage

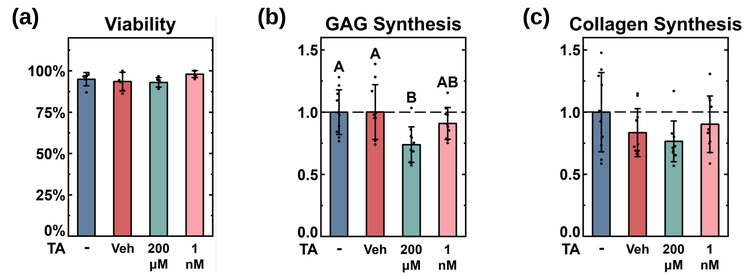


Fig. 6 Long-term effects of TA alone on human cartilage. Treated with TA alone for 14 days. TA had no significant effect on (a) chondrocyte viability or (c) collagen synthesis. (b) A saturated dose of TA did reduce GAG synthesis by~25%, but a 1 nM dose did not. Different letters indicate significant differences between groups (p<0.05).

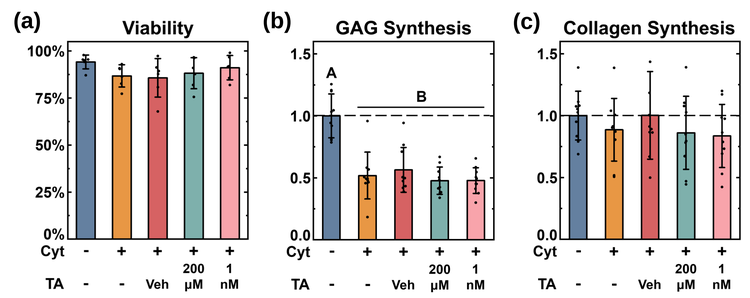


Fig. 7 Long-term effects of TA on inflammatory challenged human cartilage. Treated with TA for 14 days with simultaneous exposure to pro-inflammatory cytokine cocktail (5 ng/ml IL-1α and IL-1β, 50 ng/ml IL-6, 20 ng/ml TNF-α). Inflammatory challenge inhibited only (b) GAG synthesis, but not (c) collagen synthesis. TA had no further effect on (a) chondrocyte viability, (b) GAG synthesis, or (c) collagen synthesis in the inflamed cartilage. Different letters indicate significant differences between groups (p<0.05).

Conclusions

This study **supports intra-articular TA injections** to manage joint inflammation.

Short- and long-term exposure to TA **does not affect cell metabolic activities.**

- Chondrocyte viability, proliferation, and anabolic activities were not affected.

In contrast to pre-existing concerns, **TA inhibited GAG loss** during inflammation.

References

[1] *J. Arthroplasty*. 2021 Mar; 36(3): 845-850. [2] *Drugs*. 2019 Mar; 79: 455-462. [3] *ACS Biomat Sci Eng.* 2022 May; 8(6): 2564-2573.

Acknowledgments

This work was supported by NIH R01AR074472 (Lu), NIH COBRE P20GM139760, and NSF GRFP (Porter).