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

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INTRODUCTION: Corticosteroid injections are a commonly used method to reduce synovial inflammation of the joint [1]. However, there is a widely held fear among clinicians that steroid injections cause degradation of otherwise healthy cartilage, which leads to premature osteoarthritis (OA) [2]. Whether steroids are harmful to cartilage remains unclear with controversial evidence, depending on the OA/damage model and dose used [3]. The purpose of this study is to use our new click chemistry technique to evaluate the metabolic effects of Triamcinolone Acetonide (TA, a common corticosteroid) on *in situ* chondrocytes in young cartilage to evaluate the safety of TA injections in clinical applications.

METHODS: Cartilage tissue was harvested from the femoral condyles and patellar groove of calf knee joints (1-2 months old, 3 mm in diameter and 2 mm in thickness) and cultured in chondrogenic medium (Fig. 1) [4]. Chondrocyte metabolism and proliferation were evaluated using click chemistry techniques as described previously [5]. Samples were treated with a range of TA doses (1 nM to saturated 200 uM in DMSO). *Short-Term Effects of TA*. Cartilage samples were treated for 48 hours with TA, with and without the presence of high concentration IL-1β (10 ng/ml). Cell viability was obtained using Live/Dead™ staining (n=5). Chondrocyte proliferation (n=5), as well as the synthesis rate of new glycosaminoglycan (GAG) and collagen (n = 10) in 24 hours, were measured using click chemistry techniques. GAG and collagen synthesis were normalized to the sample weight and to control. *Long-term Effects of TA Alone*. Cartilage samples were treated with TA alone for two weeks. During the two-week culture, the longitudinal loss of GAG into the culture medium was measured every other day using the click chemistry technique and compared to control (n=10). On the final day of treatment, chondrocyte viability (n=6) and the synthesis rate of new GAG and collagen (n=10) were measured. *Effects of TA on GAG Degradation*. Cartilage samples were treated with TA in the presence of continuous low concentration IL-1β (1 ng/ml) for 10 days. Longitudinal loss of GAG from cartilage into the culture medium was measured every other day using the click chemistry technique (n=10).

RESULTS: *Short-Term Effects of TA*. A two-day culture with TA did not affect chondrocyte viability or proliferation in the top or middle zones of the cartilage samples (Fig. 2a,b). Exposure to 10 ng/ml IL-1β for two days reduced GAG synthesis by ~25% and collagen synthesis by >50% ($p < 0.05$) (Fig. 2c). Treatment with TA did not significantly rescue or worsen the GAG or collagen synthesis in the IL-1β challenged samples. (Fig. 2c). *Long-Term Effects of TA*. At all doses, a 2-week treatment with TA alone did not reduce GAG or collagen synthesis rates (Fig. 3a). Similarly, a 2-week treatment with TA alone did not cause any significant chondrocyte death in the cartilage (Fig. 3b). Treatment with TA alone for two weeks did not induce any significant GAG loss compared to control (Fig. 3c). In addition, a 1 nM dose of TA significantly reduced the GAG loss (7.5±1.2%) compared to a 200 μM dose (10.7±3.6%, $p < 0.05$) (Fig. 3c). *Effects of TA on GAG Degradation*. Exposure to continuous IL-1β for 10 days induced 28.2±7.1% GAG loss (Fig. 4). Simultaneous treatment with TA significantly reduced the amount of GAG loss to <20% in a dose dependent manner ($p < 0.05$) (Fig. 4).

DISCUSSION: Contradictory data about the effects of TA on chondrocytes have led to clinical fears about cartilage degradation. In this study, short-term TA treatment at all doses did not affect chondrocyte viability, proliferation, or extracellular matrix synthesis activities. During long-term treatment, TA did not inhibit GAG and collagen synthesis, nor did it cause any increased death of the *in situ* chondrocytes. In contrast to concerns, TA was able to prevent GAG loss caused by long-term inflammatory cytokine challenge. Over a 2-week treatment period, the 1 nM dose of TA reduced the amount of GAG loss from the cartilage compared to the 200 μM dose, suggesting a low dose of TA could still counter-act the moderate tissue degradation that occurs in an *in vitro* environment. Prior *in vitro* studies that showed TA had harmful effects were primarily conducted on monolayer cells with the clinical dose of TA for an entire joint (5-40 mg/ml). Here, when chondrocytes remain in their native matrix, the harmful effects of TA are minimal. The clinical formulation of TA injections is a suspension of practically insoluble large crystal particles (> 30 μm) [1]. The particles are too large to diffuse into the cartilage matrix, which is consistent with clinical observations that a layer of crystals deposit on the cartilage surface following injection. In this study, the highest TA dose (200 μM) was chosen based on TA's maximum solubility in a PBS:DMSO solution, which is the largest amount that could reach the *in situ* chondrocytes. The lowest TA dose (1 nM) was chosen to replicate the concentration of TA present in the joint following clinical injection with an extended-release formulation [6]. A natural future step is to investigate the physical and biochemical effects of TA deposits on the articular surface.

SIGNIFICANCE: This study supports the clinical use of intra-articular TA injections in inflamed joints with otherwise healthy cartilage.

REFERENCES: [1] *Can Assoc Radiol J*. 2019 Feb; 70(1):29-36. [2] *J Arthroplasty*. 2021 Mar; 36(3):845-850. [3] *Drugs*. 2019 Mar; 79(4):455-462. [4] *J Biomech*. 2015 Apr; 48(6):990-996. [5] *ACS Biomat Sci Eng*. 2022 May; 8(6): 2564-2573. [6] *J Control Release*. 2017 May; 253:64-72.

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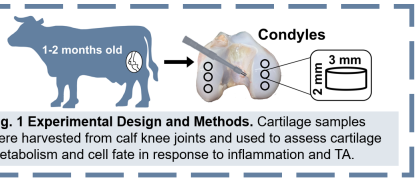

Fig. 1 Experimental Design and Methods. Cartilage samples were harvested from calf knee joints and used to assess cartilage metabolism and cell fate in response to inflammation and TA.

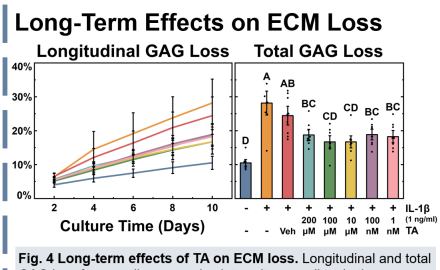

Fig. 4 Long-term effects of TA on ECM loss. Longitudinal and total GAG loss from cartilage samples into culture medium in the presence of continuous IL-1β and TA using the click chemistry technique (n = 10). Different letters indicate significant differences between groups ($p < 0.05$).

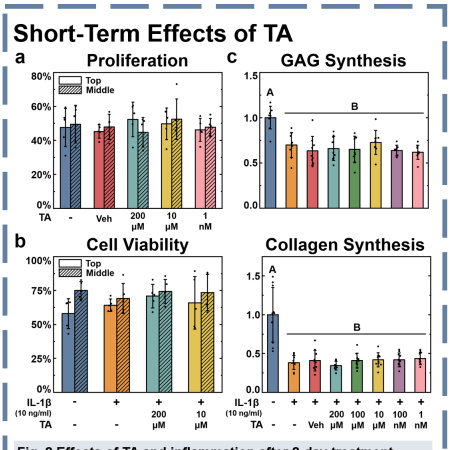

Fig. 2 Effects of TA and inflammation after 2-day treatment. Chondrocyte (a) proliferation and (b) viability in the presence of high-concentration IL-1β (10 ng/ml) (n = 10). (c) GAG and Collagen synthesis in 48 hours using click chemistry technique. Different letters indicate significant differences between groups ($p < 0.05$).

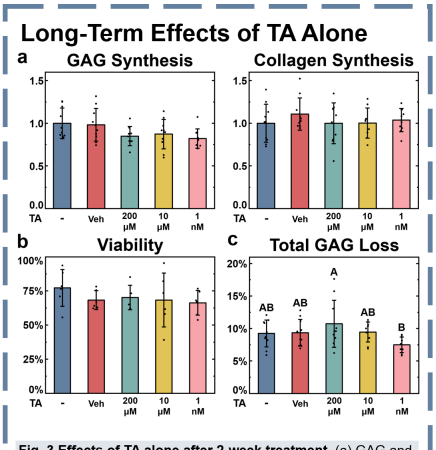

Fig. 3 Effects of TA alone after 2-week treatment. (a) GAG and collagen synthesis using click chemistry technique (n=10) and (b) chondrocyte viability after two weeks (n=6). (c) Total loss of GAG over two weeks (n=10). Different letters indicate significant differences between groups ($p < 0.05$).