Balancing Pain Relief and Cartilage Health: Differential Effects of Triamcinolone Acetonide, Lidocaine, and Bupivacaine on Human Articular Cartilage

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INTRODUCTION: Intra-articular injections are commonly used in orthopedic practice for inflammation control and pain management. Corticosteroids like triamcinolone acetonide (TA) are often administered to mitigate joint inflammation and synovitis, whereas anesthetics such as lidocaine and bupivacaine are used for rapid pain relief [1,2]. However, the long term impact of these agents on cartilage health remains controversial [3]. This study evaluated the effects of TA, lidocaine, and bupivacaine on chondrocyte viability and matrix metabolism in adult human articular cartilage.

METHODS: Human cartilage samples (3 mm diameter, 2 mm thickness) were harvested from cadaver donor knees (1F/5M, avg age 38 yrs) or total knee replacement remnants (15F/7M, avg age 65 yrs) and cultured in chondrogenic media (Fig. 1) [4]. *TA Treatment*. Cartilage samples were treated with TA at 1 nM or a saturated 200 µM in DMSO (0.087 mg/ml). To mimic intra-articular inflammation, OA samples were co-cultured with a cytokine cocktail of 5 ng/ml IL-1α, 5 ng/ml IL-1β, 50 ng/ml IL-6, and 20 ng/ml TNF-α. *Anesthetic Exposure*. Cartilage samples were submerged in 1% lidocaine (Lid) or 0.25% bupivacaine (Bup) for their *in vivo* half-lives, 90 and 160 minutes respectively (Fig. 2). Additionally, lidocaine dilutions in chondrogenic media (CG) simulated the presence of 4cc's of circulating intra-articular joint synovial fluid during injection (1:1 and 1:2 Lid:CG dilutions), as well as 1:3:4 Lid:PBS:CG to simulate saline dilution of lidocaine prior to injection. *Chondrocyte Viability*. Cell viability was measured using Live/Dead^{TM} assay following 14-day TA treatment or 24-hour anesthetic exposure. *Gene Expression*. Following 48-hour TA treatment, RNA expression levels of MMP13, ADAMTS5, ACAN, and COL2A1 were quantified with qRT-PCR, and the gene expression fold change was analyzed using the $2^{-\Delta\Delta C(T)}$ method after data was normalized to the reference gene β-Actin. *ECM Synthesis and Degradation*. A high-resolution click-chemistry assay quantified glycosaminoglycan (GAG) and collagen synthesis in cartilage and tracked GAG loss from the tissue [5]. Cartilage samples were treated with TA for a continuous 14 days. Healthy cartilage samples were also treated for 2 days, followed by a 14-day recovery in chondrogenic media. During the final four days of culture, synthesis of nascent GAG and collagen in the tissue was measured using click chemistry. During a continuous 18 day treatment with TA, the longitudinal loss of GAG from cartilage samples into the culture media was measured every other day.

RESULTS: *Triamcinolone Acetonide*. TA exhibited no chondrotoxicity at either 1 nM or 200 µM, even after prolonged 14-day treatment in healthy (Fig. 3a) and OA human cartilage (Fig. 3b,c). TA downregulated catabolic gene expression (MMP13, ADAMTS5) in healthy and OA tissue, and only minimally inhibited COL2A1 expression in healthy human cartilage (Fig. 4a,b). Treatment with TA did not induce any further ECM degradation in healthy or OA cartilage (Fig. 5a,b), but co-treatment with TA was not able to reduce cytokine-induced GAG loss (Fig. 5c). After 14 day treatment, high-dose TA (200 µM) modestly reduced GAG synthesis in healthy and OA cartilage, and collagen synthesis only in healthy tissue (Fig. 6a, Fig. 7a). A 1 nM dose of TA only had a minimal impact on collagen synthesis in healthy cartilage (Fig. 7a). A short-term exposure followed by recovery showed full restoration of anabolic activity in healthy cartilage (Fig. 6b). Exposure to pro-inflammatory cytokines reduced only GAG synthesis in OA cartilage, and TA at either dose had no further effect (Fig. 7b). *Lidocaine and Bupivacaine*. In both healthy and OA human cartilage, submersion in lidocaine and bupivacaine led to nearly 100% cell death across the entire tissue depth (Fig. 8a,b). When lidocaine was diluted to simulate clinical practice, cell viability improved in a dose-dependent manner. At a 1:3:4 Lid:PBS:CG dilution, cell viability was not significantly different from the PBS control in the top and middle region (Fig. 9a,b).

DISCUSSION: Triamcinolone acetonide and local anesthetics exhibit opposing effects on cartilage health. TA was well tolerated by both healthy and OA human cartilage, even at supraphysiological concentrations [6], with minimal suppression of matrix synthesis and no evidence of toxicity or degradation. In contrast, when lidocaine and bupivacaine were applied undiluted, they are highly chondrotoxic. However, lidocaine-induced toxicity was substantially reduced when diluted, particularly at the 1:3:4 dilution. **CLINICAL RELEVANCE:** Intra-articular anesthetics should be administered with caution due to their potential chondrotoxicity, and to reduce toxicity, lidocaine should be diluted prior to intra-articular injection. Corticosteroid injections, by contrast, appear safe for both single and repeated use in human cartilage, providing effective inflammation control without direct cartilage damage.

REFERENCES: [1] Blankstein+ 2021. [2] MacMahon+ 2009. [3] Guermazi+ 2023. [4] Zhou+ 2015. [5] Porter+ 2022. [6] Porter+ 2024.

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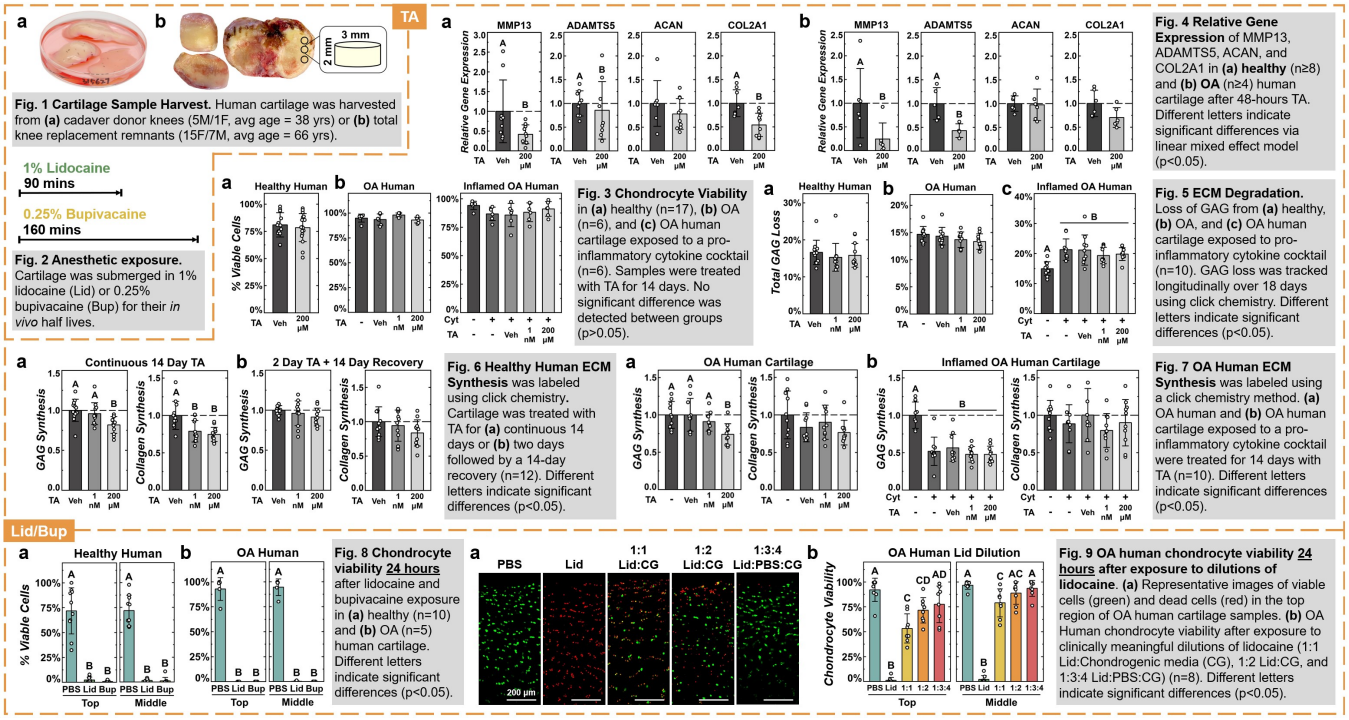

Fig. 1 Cartilage Sample Harvest. Human cartilage was harvested from **(a)** cadaver donor knees (5M/1F, avg age = 38 yrs) or **(b)** total knee replacement remnants (15F/7M, avg age = 66 yrs).

Fig. 2 Anesthetic exposure. Cartilage was submerged in 1% lidocaine (Lid) or 0.25% bupivacaine (Bup) for their *in vivo* half lives.

Fig. 3 Chondrocyte Viability in **(a)** healthy (n=17), **(b)** OA (n=6), and **(c)** OA human cartilage exposed to a pro-inflammatory cytokine cocktail (n=6). Samples were treated with TA for 14 days. No significant difference was detected between groups (p>0.05).

Fig. 4 Relative Gene Expression of MMP13, ADAMTS5, ACAN, and COL2A1 in **(a) healthy** (n≥8) and **(b) OA** (n≥4) human cartilage after 48-hours TA. Different letters indicate significant differences via linear mixed effect model (p<0.05).

Fig. 5 ECM Degradation. Loss of GAG from **(a)** healthy, **(b)** OA, and **(c)** OA human cartilage exposed to pro-inflammatory cytokine cocktail (n=10). GAG loss was tracked longitudinally over 18 days using click chemistry. Different letters indicate significant differences (p<0.05).

Fig. 6 Healthy Human ECM Synthesis was labeled using click chemistry. Cartilage was treated with TA for **(a)** continuous 14 days or **(b)** two days followed by a 14-day recovery (n=12). Different letters indicate significant differences (p<0.05).

Fig. 7 OA Human ECM Synthesis was labeled using a click chemistry method. **(a)** OA human and **(b)** OA human cartilage exposed to a pro-inflammatory cytokine cocktail were treated for 14 days with TA (n=10). Different letters indicate significant differences (p<0.05).

Fig. 8 Chondrocyte viability 24 hours after lidocaine and bupivacaine exposure in **(a)** healthy (n=10) and **(b)** OA (n=5) human cartilage. Different letters indicate significant differences (p<0.05).

Fig. 9 OA human chondrocyte viability 24 hours after exposure to dilutions of lidocaine. (a) Representative images of viable cells (green) and dead cells (red) in the top region of OA human cartilage samples. **(b)** OA Human chondrocyte viability after exposure to clinically meaningful dilutions of lidocaine (1:1 Lid:Chondrogenic media (CG), 1:2 Lid:CG, and 1:3:4 Lid:PBS:CG) (n=8). Different letters indicate significant differences (p<0.05).