Attenuating Collagen Deposition by Synoviocytes from Osteoarthritic Patients with Synovial Fibrosis

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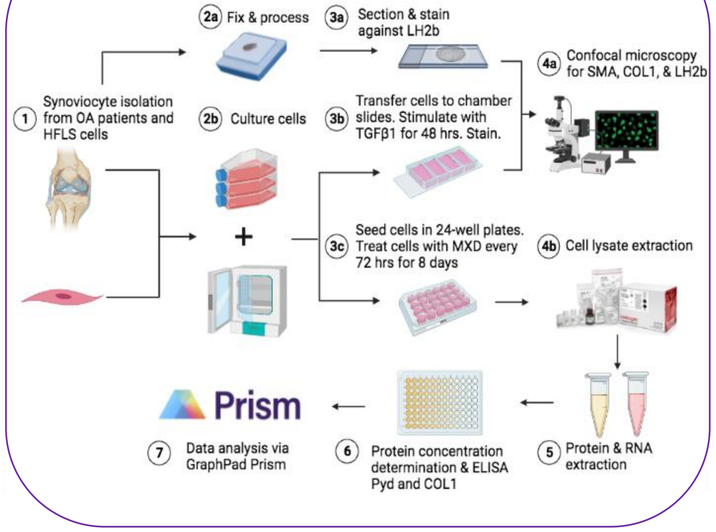
Introduction

- One in five Americans suffer from osteoarthritis (OA), an articular disease characterized by cartilage degradation and inflammation (1). A hallmark of OA is fibrosis, or deposition of stiff matrix mostly composed of type-I collagen (COL1), which can impair the function of cardiovascular, renal, pulmonary, and especially musculoskeletal systems (2, 3).
- COL1 is synthesized and deposited largely by myofibroblasts and mostly driven by the receptor-mediated transforming growth factor (TGF)β1-ALK5-Smad2/3 cascade. Stimulation of naïve fibroblasts with TGFβ1 induces proliferation, smooth muscle actin (SMA)+ myofibroblast differentiation, and COL1 synthesis (4).
- The COL1 triple helix undergoes modifications, such as lysine to hydroxylysine conversion by Procollagen-Lysine,2-Oxoglutarate 5-Dioxygenase 2 (*Plod*)*2*-encoded lysyl hydroxylase (LH)2b to catalyze pyridinoline (Pyd) cross-links (Fig.1) that strengthen COL1 fibrils (4).
- Synovial fibrosis (SFb) in OA is characterized by aberrant COL1 deposition in the subintima of the synovium encapsulating the joint (2). Our lab has generated evidence that LH2b levels correspond to severity of SFb, suggesting that OA patients classified with high fibrosis have an abundance of Pyd cross-links that are highly resilient to degradation by matrix metalloproteases (3).
- Minoxidil (MXD; Fig. 2) is an FDA-approved drug for hypertension and alopecia. Recently, MXD has been shown to interfere with LH2b transcription, consequently weakening fibrous COL1 by limiting over-hydroxylation of telopeptides and decreasing Pyd cross-links. Notably, MXD functions intracellularly and resultantly does not disrupt COL1 already deposited into the extracellular matrix, making it a safe and attractive therapeutic (5, 6, 7).
- Our goal is to test MXD's potential as an anti-fibrotic agent by applying it to cultured fibroblastic synovial cells (FSCs) from knee OA patients and a human fibroblast-like synoviocyte (HFLS) cell line to evaluate attenuation of Pyd cross-links which would weaken fibrous collagen deposits.

Research Question

Can MXD, a proposed LH2b antagonist, effectively attenuate Pyd cross-links and thus weaken fibrous COL1 deposited by OA FSCs?

Methods



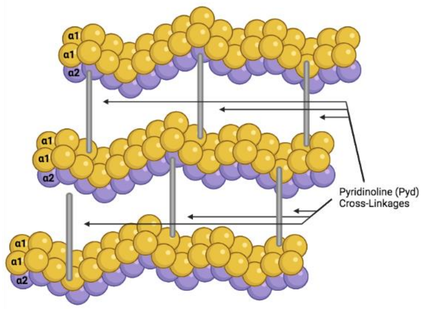


Figure 1: Structure of COL1 with Pyd

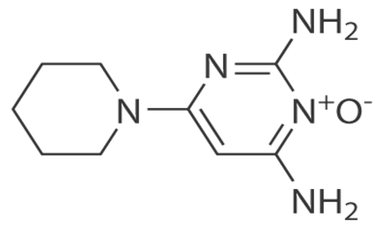


Figure 2: Structure of MXD

Pilot Results

- SMA and COL1 are proportionally upregulated by increasing concentrations of exogenous TGFβ1 in HFLS cells.
- LH2b expression is significantly elevated in the synovium of OA patients presenting with high SFb compared to a low fibrosis cohort.

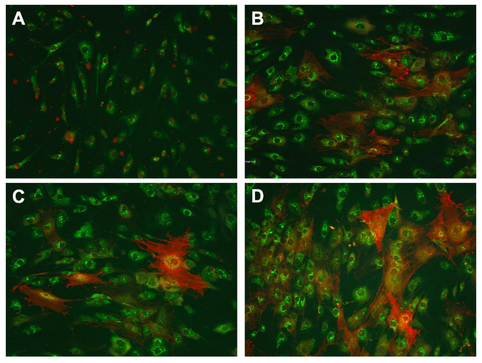


Figure 3: SMA (red) and COL1 (green) expression with increasing concentrations of TGFβ1. A = 0 ng/ml; B = 1 ng/ml; C = 2 ng/ml; D = 4 ng/ml.

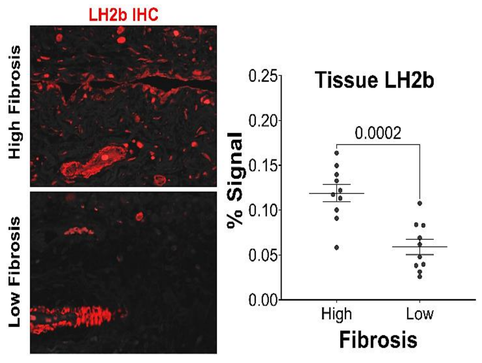


Figure 4: LH2b expression in the synovium of FSCs isolated from high SFb (0.12±0.03) and low SFb (0.06±0.03) patients.

- Total COL1 deposition appears to be similar after MXD administration to OA patient FSCs.
- Total Pyd cross-link content appears to decrease with MXD in OA patient FSCs.

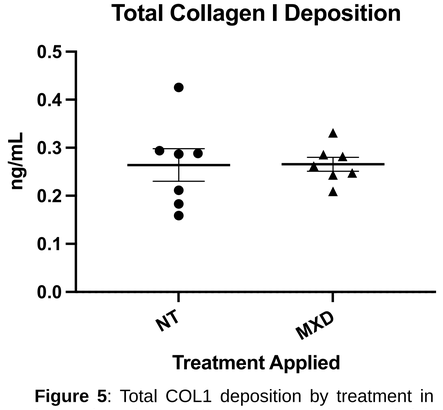


Figure 5: Total COL1 deposition by treatment in isolated patient FSCs measured by sandwich ELISA.

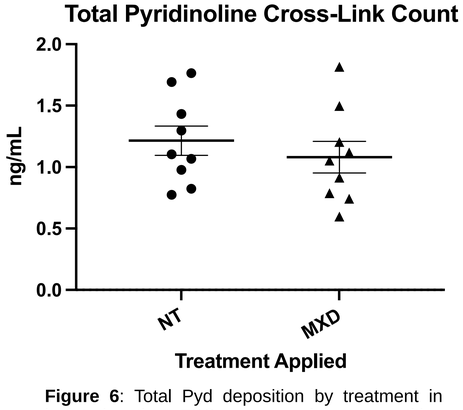


Figure 6: Total Pyd deposition by treatment in isolated patient FSCs measured by competitive ELISA.

Findings

- SMA and COL1 expression are proportionally upregulated with increasing concentrations of TGFβ1 in HFLS cells (Figure 3). Increased expression of SMA demonstrates FSC differentiation into myofibroblasts.
- LH2b expression is greatly increased in the synovium of OA patients classified with high SFb compared to those with low SFb (Figure 4).
- Total COL1 deposition appears to be similar across treatments in patient FSCs ($p < 0.9628$, Figure 5).
- Although not significant, total Pyd measurements in patient FSCs treated with MXD are generally lower than untreated FSCs ($p < 0.4560$, Figure 6).

Discussion

- Although MXD has been recently suggested as an antifibrotic agent that interferes with the transcription of *Plod2* and thus translation of LH2b (5, 6, 7), our approach needs a higher sample size to conclude a significant effect. Considering the higher LH2b expression presented by patients with high SFb and understanding OA pathophysiology is influenced by inflammatory status, sex, and race (8), it is presumed that the effect of MXD could be amplified once data are adjusted for various confounders.
- Taken together, our pilot data suggests the potential inability of MXD to interfere with COL1 synthesis but a possibility that it compromises post-translational Pyd cross-linking in OA patient FSCs.

Limitations

- Our sample size limits the significance of a potential MXD effect on Pyd metrics.
- Primary FSCs are difficult to expand, so cell lysates were generated from suboptimal sample volumes, which require more precise/sensitive assays and standard readings.
- Lack of hydroxyproline assays to validate MXD effects on Pyd (7).

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