Targeted Matrix Metalloproteinase (MMP-2,-3,-7) Inhibition Maintains Matrix Architecture and Function in Osteoarthritic Cartilage

Marina Danalache[1], Moritz Kurschatke[1], Elena Pradarutti[1], Rosa Riester[1], Ulf Krister Hofmann[2], Klaus Peter Günther[1], Farshid Guilak[3,4]
[1]Department of Orthopedic Surgery, University Hospital of Tübingen, Tübingen 72072, Germany.
[2] Department of Orthopedic, Trauma, and Reconstructive Surgery, RWTH Aachen University Hospital, Aachen 52074, Germany
[3]Department of Orthopedic Surgery, Washington University, St. Louis, MO 63110, USA;
[4]Shriners Hospitals for Children, St. Louis, MO 63110, USA.
Marina.Danalache@med.uni-tuebingen.de

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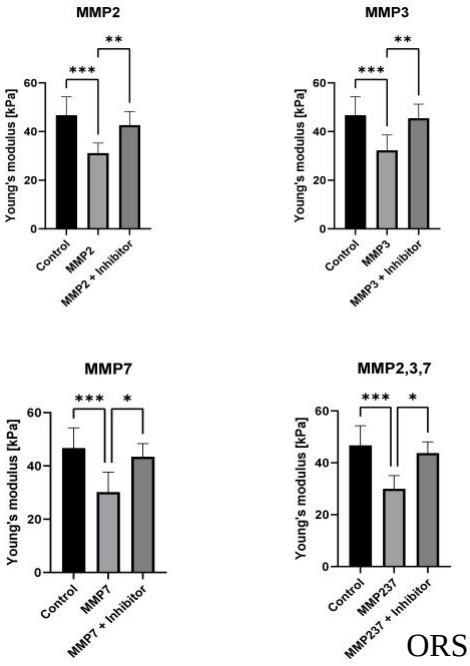
INTRODUCTION: Osteoarthritis (OA) is one of the most prevalent degenerative joint diseases, characterized by the progressive degradation of articular cartilage. A hallmark of OA pathophysiology is the breakdown of both the extracellular matrix (ECM) and the pericellular matrix (PCM). The PCM is a highly specialized region that immediately surrounds all chondrocytes and serves as a crucial mechanotransductive and mechanoprotective interface within cartilage tissue. In this regard, alterations in PCM structure and mechanical properties have been identified as one of the first hallmarks of OA and are hypothesized to result in dysfunctional cell-matrix interactions that can further exacerbate loss of chondrocyte homeostasis. Matrix metalloproteinases (MMPs) are central mediators of matrix remodeling and degradation in OA. Our previous findings identified MMP-2, -3, and -7 as key drivers of early PCM destruction [1]. This study aimed to evaluate the impact of targeted pharmacological inhibition of these MMPs on preserving the structural and mechanical integrity of the PCM, while also assessing therapeutic efficacy and potential adverse effects on the surrounding ECM.

METHODS: Human cartilage explants (0.3mmx4mm), resected from macroscopically intact regions of the femoral condyles (N= 26; median age: 64 years, range 50-87; 16 males, 10 females), were allocated to nine experimental groups. Explants were exposed to MMP-2, MMP-3, or MMP-7 individually, or to a combination of all three enzymes, followed by treatment with selective, pharmacologically available small-molecule inhibitors targeting MMP-2, MMP-3, or MMP-7, administered either individually, in pairwise combinations, or as a triple-inhibition cocktail. The concentrations of exogenously applied MMPs were calibrated to levels previously identified in early stage OA [1]. The inhibitory efficacy of each compound was validated via enzymatic activity assays quantifying residual MMP activity post-treatment. The structural integrity of the PCM and the surrounding ECM was assessed both qualitatively using immunolabelling and quantitatively via targeted immunoassays. To capture biomechanical alterations, we employed immunofluorescence-guided atomic force microscopy (AFM) in contact mode, enabling microscale, site-specific elasticity assessment of both PCM and ECM.

RESULTS: Treatment with specific inhibitors resulted in a significant reduction of MMP-induced degradation, with combined inhibition of MMP-2, -3, and -7 achieving an overall MMP activity reduction of 80%. Notably, inhibition of MMP-2, -3, and -7 promoted preservation of key PCM components, particularly collagen type VI and perlecan, as well as maintenance of the overall ECM structural integrity. Enzymatic degradation of cartilage PCM by individual MMPs significantly reduced tissue stiffness, as indicated by a decrease in the Young's modulus across all treatment conditions. Exposure to MMP-2 resulted in a marked reduction in Young's modulus compared with control samples ($p < 0.01$), an effect that was partially rescued by selective MMP-2 inhibition ($p < 0.01$ vs. MMP-2 alone). Similarly, MMP-3 treatment significantly lowered tissue stiffness relative to controls ($p < 0.01$), with MMP-3 inhibition restoring the Young's modulus to near-control levels ($p < 0.05$ vs. MMP-3 alone). MMP-7 exposure caused a pronounced reduction in Young's modulus ($p < 0.001$ vs. control), and inhibition of MMP-7 significantly mitigated this stiffness loss ($p < 0.01$ vs. MMP-7 alone). Simultaneous exposure to MMP-2, MMP-3, and MMP-7 induced a substantial decline in Young's modulus ($p < 0.001$ vs. control), whereas the triple-inhibition strategy restored mechanical properties to levels significantly higher than those observed with combined MMP treatment ($p < 0.05$) and comparable to control values (Fig.1). These findings demonstrate that selective inhibition of MMP-2, MMP-3, and MMP whether individually or in combination attenuates MMP-induced mechanical weakening of articular cartilage.

DISCUSSION: These findings highlight the therapeutic potential of selective MMP inhibition in mitigating cartilage matrix degradation. The substantial reduction in MMP activity particularly through inhibition of MMP-2, -3, and -7 is associated with the preservation of essential PCM components, both of which are vital for maintaining chondrocyte function and matrix homeostasis. The observed increase in PCM stiffness further underscores the mechanical benefits of targeted inhibition, suggesting a direct link between matrix preservation and functional tissue resilience. By protecting also structural integrity of the ECM, this strategy effectively restores mechanical properties to near-native levels, reinforcing the role of MMP modulation in cartilage preservation.

SIGNIFICANCE: Targeted inhibition of MMP-2, -3, and -7 significantly preserves cartilage matrix integrity by reducing enzymatic degradation, maintaining PCM and ECM components, and restoring mechanical stiffness to near-native levels. These findings underscore the therapeutic potential of selective MMP modulation as a strategy to prevent or slow down slow cartilage degeneration, mitigate disease progression, and sustain joint function.



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FIGURES:

Fig 1. MMP inhibition restores the biomechanical properties of the PCM. Articular cartilage samples were treated with MMP-2, MMP-3, and MMP-7 individually and in combination, with or without their respective pharmacological inhibitors. The stiffness (Young's moduli) of the PCM was quantitatively assessed using immunofluorescence-guided atomic force microscopy (AFM). MMP treatment alone led to a significant reduction in PCM stiffness across all groups. In contrast, treatment with MMP inhibitors, either individually or in combination resulted in a marked recovery of PCM stiffness to levels comparable to those observed in untreated, healthy control samples. *p<0.05, **p<0.01, ***p<0.001