

A2M Attenuates Cartilage Degeneration by Binding to and Blocking the IL-1R1 Cascade in a Large Preclinical Pig Model

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INTRODUCTION: Inflammation plays a critical role in posttraumatic osteoarthritis (PTOA). The binding of IL-1 to IL-1R1 initiates the inflammation cascade and induces cartilage degeneration. A2-Macroglobulin (A2M), is a broad-spectrum anti-inflammatory protein with the potential to block inflammatory pathways contributing to PTOA development. We hypothesized that A2M would reduce cartilage damage and synovitis by binding to and blocking IL-1R1 pathway in the previously developed modified intra-articular drilling (mIAD) minipig model, which isolates the PTOA-inducing effects of inflammation from mechanically-driven tissue damage.

METHODS: 48 Yucatan minipigs were randomized into four groups (n=12 each): (1) mIAD+saline, (2) mIAD and one intra-articular A2M injection (mIAD+A2M-1), (3) mIAD and three A2M injections (mIAD+A2M-3), and (4) sham arthrotomy control. The mIAD procedure involved drilling two osseous 2mm diameter tunnels 15mm deep into each of the tibia and femur adjacent to the ACL attachment sites. Surgical hind limbs were harvested 15 weeks post-surgery. Cartilage integrity was evaluated using macroscopic and microscopic scoring systems. Synovium changes were assessed using microscopic scoring systems. IL-1R1, IL-1 β , NF- κ B, TNF- α , and MMP-13 mRNA expression levels in the cartilage were measured using RT-PCR. IL-1R1 and its binding to A2M were determined by immunohistochemistry on cartilage. A2M was labeled with VivoTag™ 680 (red).

RESULTS: We have reported that the mIAD+A2M-1 and mIAD+A2M-3 had significantly less macroscopic and microscopic cartilage degeneration compared to the mIAD+saline group but similar damage compared with the sham group¹. In the cartilage, double-labeling immunofluorescence analysis showed that IL-1R1 was localized (green) at the area of cartilage degeneration, and A2M labeled with VivoTag™ 680 (Red) was colocalized with IL-1R1 positive cells. A2M and IL-1R1 bindings were demonstrated as evidenced by the fluorescent overlapping signaling (Orange) (Fig.1). The chondrocytes with the overlapping signaling were as much as 36% in the surface fibrillation, much higher than those in the normal surface in quantitative analysis (Figure 1). A2M reduced the levels of PTOA-related genes, such as IL-1R1, IL-1 β , MMP-13, and TNF- α , in cartilage compared with the mIAD+saline group (Fig.2). We further demonstrated that blockage of IL-1R1 with A2M reduced the levels of inflammation induced by IL-1.

DISCUSSION: Previous and current studies demonstrated that A2M treatment successfully reduces cartilage degeneration, synovitis,¹ and markers of inflammation in both the synovium and cartilage compared to animals that had undergone mIAD without treatment. The present results showing colocalization of A2M and IL-1R1 in areas of cartilage lesions suggests another potential anti-inflammatory mechanism of A2M. In addition to directly binding to free IL-1 β , A2M may directly bind to the IL-1 β receptor, limiting the extent of PTOA progression.

SIGNIFICANCE/CLINICAL RELEVANCE: These data suggest that α_2 M may effectively attenuate PTOA cartilage damage by binding to and blocking IL-1R1 pathway, inhibiting the catabolic cascade initiated by inflammatory mediator IL-1R1. Thus, targeting IL-1R1 by A2M may be a novel therapy for PTOA patients.

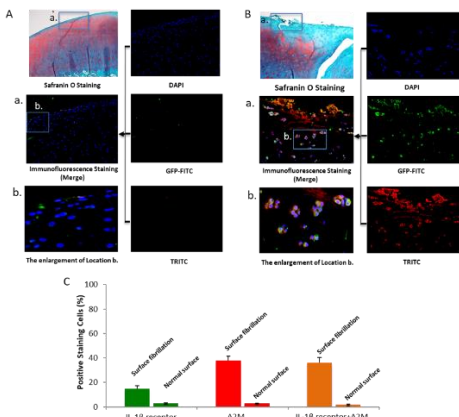


Fig.1. Double labeling immunofluorescent analysis and safranin O staining reveals that (A) there are no IL-1R1 positive cells detected in the normal cartilage, and (B) A2M bindings to IL-1 β receptor-positive cells in the surface of OA cartilage. A2M was labeled with VivoTag™ 680 (Red) and IL-1 β receptor immuno-stained by the secondary antibody Alex Fluor Plus 488 (Green). A2M and IL-1 β receptor bindings were demonstrated by the fluorescent overlapping signaling (Orange). (C) The cells with the overlapping signaling are as much as 36% in the surface fibrillation, much higher than those in the normal surface in quantitative analysis.

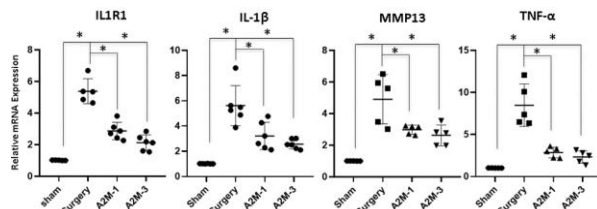


Fig.2. Inflammatory gene expression levels from cartilage. The relative mRNA expressions of IL1R1, IL-1 β , MMP13 and TNF- α was significantly higher in the untreated surgical animals compared with A2M treated animals ($p < 0.05$).

REFERENCES: 1. Changqi Sun et al. 2023 ORS