Identification Of Alpha 2 Macroglobulin (a2m) As A Master Inhibitor To Attenuate Post-traumatic Osteoarthritis Cartilage Degeneration

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Introduction: Since elevated levels of catabolic enzymes in synovial fluid appear to induce chondrocyte death and cartilage matrix degeneration within one week of injury 1,2, early intervention strategies should focus on modulating these cartilage degrading enzymes within this time frame. Alpha-2 macroglobulin (A2M) inhibits all classes of endoproteases, it could be used to slow down the cartilage damage that follows traumatic knee injuries by neutralizing cartilage catabolic enzymes. This study is to determine if supplemental intra-articular alpha-2 macroglobulin (A2M) has a chondroprotective effect in anterior cruciate ligament-transected (ACLT) knees.

Methods: A2M was identified as a potential therapeutic agent by comparing A2M concentrations in serum, synovial fluid (SF), and cartilage from normal and osteoarthritic (OA) patients by Western blotting, mass spectrometry, ELISA, and immunohistochemistry (IHC). The effects of A2M on IL-1-induced cartilage catabolic enzymes were evaluated by Luminex and ELISA in cultured chondrocytes and cartilage organ cultures. In vivo effects were evaluated in male rats (N=120) randomized to four treatments: (1) ACLT + saline, (2) ACLT + A2M (1IU/kg), (3) ACLT + A2M (2IU/kg) or (4) sham surgery + saline. Intra-articular injections were given immediately and 3 days after surgery, then once weekly for 6 weeks. Catabolic enzymes were monitored in vivo via Fluorescence Molecular Tomography (FMT) using murine partial medial meniscectomy (PMM). Histological analyses and IHC were performed to assess cartilage damage. The concentration of MMP-13 in SF lavages was measured using ELISA. Gene expression was quantified by RT-qPCR.

Results: The levels of total A2M were 7-fold lower, while the levels of MMP-13 were 2.8-fold higher in SF compared with serum from OA patients (Fig.1A). However, the level of inactive A2M in SF was higher than that in serum. Supplementation with exogenous A2M inhibited cartilage catabolic enzymes in a dose-dependent manner in human chondrocytes (Fig. 1B). Catabolic enzymes in PMM mice peaked 2 days after surgery (Fig. 2). Early supplemental intra-articular injection of A2M reduced the concentration of MMP-13 in SF and attenuated OA pathogenesis in the rat ACLT model (Fig. 3). RT-qPCR indicated that supplemental intra-articular A2M inhibits catabolism and enhances anabolic metabolism.

Figure 1. A2M negatively regulates cartilage catabolic cytokines and MMPs. (A) Higher A2M concentration and lower MMP-13 content were detected in the serum, when compared with OA synovial fluid (same patients. N=20). (B) MMP-13 activity was induced by IL-1 (10 ng/ml), and inhibited by A2M in a dose-dependent manner in human OA chondrocytes.

Figure 2. The peak of cartilage catabolic factors occurs at day 2 after knee joint injury. The highest catespin activity during the 9 week period after joint injury was detected in 2 days after surgery (A-a), indicating the peak of the inflammatory response occurs right after surgery.

Figure 3. Supplemental intra-articular A2M attenuated PTOA pathogenesis in a rat ACLT model. (A) Decreased cartilage damage with stronger Safranin-O staining was detected in the articular cartilage of A2M-treated animals comparing to the untreated controls. (B) OARSI score (Mean±SD) indicated that the cartilage damage in the ACLT + Saline group was the highest of all the groups, while cartilage in the Sham + Saline group had the least damage. Cartilage damage in the ACLT + A2M (1IU/kg) group was more than in the ACLT + A2M (2IU/kg) group. (C) Similar to the Sham group, A2M-treated groups had a lower MMP-13 concentration in synovial fluid than that in the ACLT+Saline group.

Discussion: A2M is a plasma protease inhibitor that is not present in sufficient concentrations to inactivate the high concentrations of catabolic enzymes found in OA SF although the level of A2M in plasma is much higher than that in SF. This difference is thought to be due to the large molecular weight of A2M, which prevents it from migrating into the SF. Our in vitro and in vivo data further indicate A2M is a potential candidate to prevent OA pathogenesis by reducing cartilage catabolic enzymes.

Significance: Our findings suggest that early supplemental intra-articular A2M could provide chondral protection for post traumatic OA.

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