

Introduction

Objective: To test the hypothesis that the addition of proinflammatory cytokines or cartilage-degrading metalloproteinases (ADAMTS and MMP) stimulate cartilage degradation that will be inhibited by A2M.

Design: Controlled in vitro cartilage degradation assay.

Setting: Tissue culture.

Participants: Bovine Cartilage Explants (BCE) in culture media.

Interventions: We treated BCE with proinflammatory cytokines (TNF α or IL-1 β) or cartilage-degrading metalloproteinases (ADAMTS-5, ADAMTS-4, MMP-7, or MMP-12) in the presence or absence of purified A2M.

Main Outcome Measures: Cartilage catabolism is measured following 2 days in culture by 1) proteoglycan release via the presence of sulfated glycosaminoglycan (sGAG) and 2) the presence of Aggrecan G3 fragments by Western blotting.

Methods

Bovine articular cartilage explants (BCE, 100 \pm 4 mg) are isolated from 1 - 1.5 year-old heifers and are equilibrated 3 days in culture. To degrade cartilage by protease digestions, BCE is incubated 2 days in Serum-free Media (SFM) with or without 500ng/ml ADAMTS-4 or ADAMTS-5 and 3 - 5 μ g/ml of MMP-3, MMP-7, MMP-12, or MMP-13. MMP-3 was activated with chymotrypsin before application on BCE. For cytokine-induced cartilage catabolism, BCE (200 \pm 4 mg) is incubated 3 days in SFM with or without 80ng/ml human TNF- α and 8ng/ml human IL-1 β . Cartilage degradation is inhibited with the addition of 100 μ g/ml of purified human A2M for protease digestions or 5mg/ml A2M for cytokine-induced degradation. Cartilage catabolism is measured in culture supernatant by 1) proteoglycan release via the presence of sulfated glycosaminoglycan (sGAG) and 2) the presence of cartilage proteoglycan fragments by Bio-Rad Stainless SDS-PAGE and Aggrecan G3 fragments by Western blotting. Fibronectin and Aggrecan Complexes (FAC) are formed by combining degraded cartilage matrix proteoglycans from the BCE experiments with Fibronectin and Synovial Fluid and incubating for 4 hours. Newly formed FAC is measured by the FACT ELISA, with the alteration of using an α -Aggrecan G3 antibody needed to recognize bovine aggrecan.

A2M inhibits Cartilage Catabolism

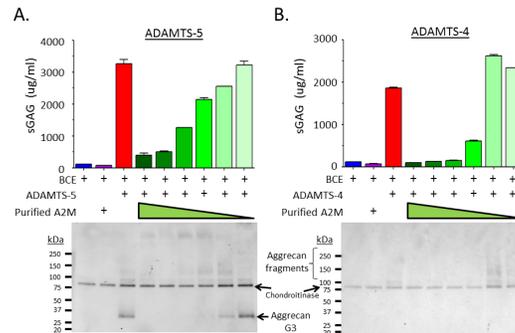
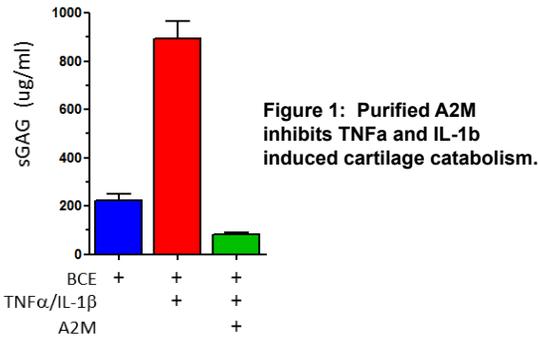


Figure 2: ADAMTS-5 and ADAMTS-4 degradation of cartilage produces an Aggrecan G3 fragment, which is inhibited by purified A2M in a dose dependent manner.

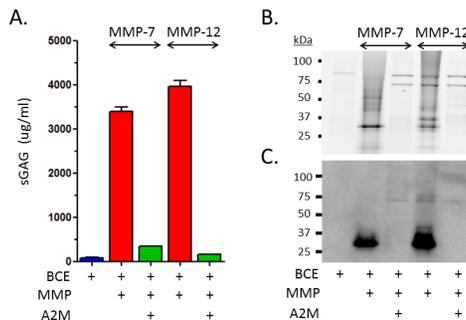


Figure 3: MMP-7 and MMP-12 degradation of cartilage produces an Aggrecan G3 fragment, which is inhibited by purified A2M.

Results

Cartilage degradation in 100mg BCE was induced by addition of cytokines (80ng/ml TNF- α or 8ng/ml IL-1 β) or matrix metalloproteinases (500ng/ml ADAMTS-4 or ADAMTS-5; 5 μ g/ml MMP-7 or MMP-12) and resulted in increased sGAG and Aggrecan G3 fragments in the medium. Addition of 100 μ g/ml A2M inhibited cartilage catabolism induced by metalloproteinases in a dose dependent manner. The IC₅₀ needed to inhibit cartilage catabolism by 500ng/ml proteases was 7 μ g/ml A2M for ADAMTS-5 and 3 μ g/ml for ADAMTS-4. Addition of 5mg/ml A2M also inhibited cartilage catabolism induced by TNF- α or IL-1 β . Further, A2M blocked production of Aggrecan G3 fragments, which form complexes with fibronectin and are a marker for pain and degrading joints.

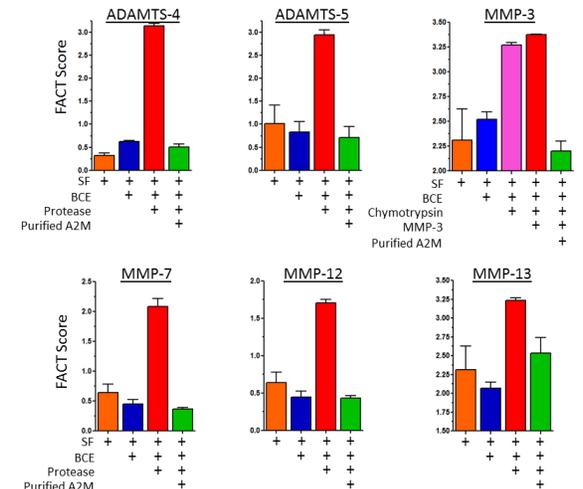


Figure 4: Prevention of cartilage catabolism by A2M treatment further prevent formation of the Fibronectin Aggrecan Complex, a marker for knee pain and cartilage degradation.

Conclusions

Osteoarthritis (OA) is characterized by progressive degeneration of articular cartilage. The BCE model is representative of studying putative therapeutics in OA. This study demonstrates A2M may protect against cartilage loss.

Disclosure

All authors have Cytonics shares or stock options.