Can Cartilage Degradation be Prevented by Platelet Rich Plasma (PRP) Preparations on Bovine Cartilage Explants?

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Introduction

Objective: To test the hypotheses that cartilage catabolism caused by proinflammatory cytokines and cartilage-degrading metalloproteinases (ADAMTS) can be inhibited by preparations of Leukocyte-rich PRP (LR-PRP) or Autologous Platelet Integrated Concentrate (APIC-PRP).
Design: Controlled in vitro cartilage degradation assay.
Setting: Tissue culture.
Participants: Bovine Cartilage Explants (BCE) in culture media.
Interventions: BCE was treated with ADAMTS-5, TNF-α or IL-1β in the presence or absence of LR-PRP or APIC-PRP.
Main Outcome Measures: Cartilage catabolism is measured following 2 or 3 days in culture by proteoglycan release via the presence of sulfated glycosaminoglycan (sGAG) in the media.

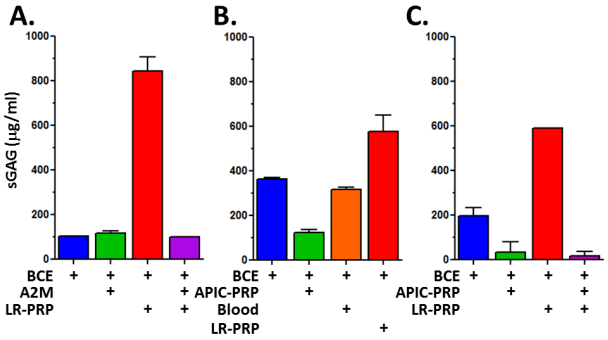


Figure 2: Leukocyte-rich Platelet Rich Plasma (LR-PRP) (A, B, C), but not blood (B) induces cartilage catabolism. Treatment with either A2M (A) or APIC-PRP (B, C) inhibits cartilage degradation.

Methods

Bovine articular cartilage explants (BCE, 200 ±4 mg) are isolated from 1 - 1.5 year-old heifers and are equilibrated 3 days in culture. BCE cultures were treated for 3 days with or without a 33% (v/v) Leukocyte-rich Platelet-rich Plasma (LR-PRP), blood, or APIC-PRP prepared from the same patient. Protease digestion of cartilage with 500 ng/ml ADAMTS-5 for 2 days was inhibited with a 2-fold serial dilution of APIC-PRP [ED_{50} = 0.1% v/v]. For cytokine-induced cartilage catabolism, BCE is incubated 3 days in SFM with or without 80ng/ml human TNF-α or 8ng/ml human IL-1β. Cartilage degradation is inhibited with the addition of 5 mg/ml A2M or 30% (v/v) APIC-PRP. To demonstrate a dose-response curve of APIC-PRP, 3-fold serial dilutions of APIC-PRP [ED_{50} = 3% v/v] were used to inhibit TNF-α/ IL-1β induced cartilage degradation. Cartilage catabolism is measured in culture supernatant by proteoglycan release via the presence of sulfated glycosaminoglycan (sGAG) using a DMMB assay with chondroitin sulphate standard curve.

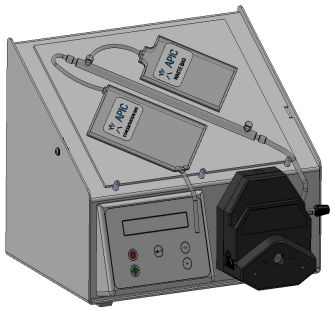


Figure 1: The APIC-PRP system uses centrifugation and filtration in a unique two-stage process to concentrate platelets and A2M and remove White Blood Cells.

APIC-PRP inhibits Cartilage Catabolism

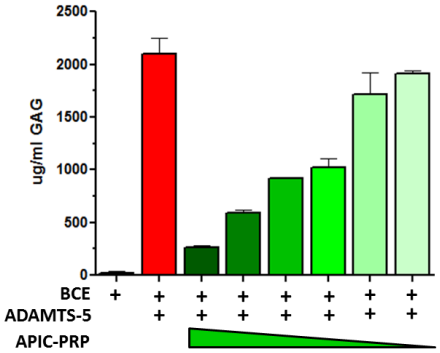


Figure 4: APIC-PRP inhibits ADAMTS-5 induced cartilage catabolism in a dose dependent manner.

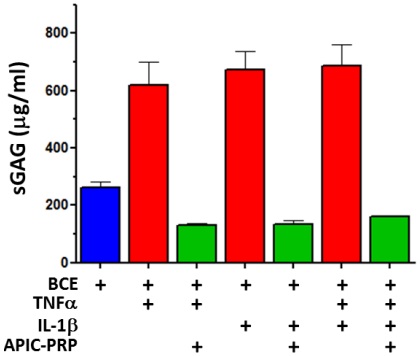
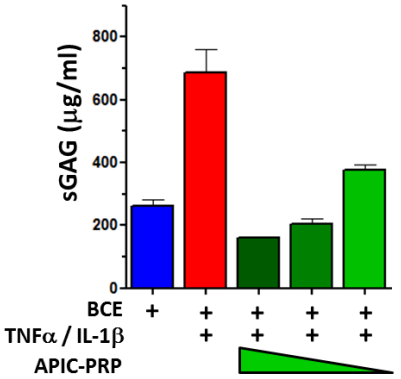


Figure 4: APIC-PRP efficiently inhibits cartilage catabolism induced by pro-inflammatory cytokines TNF-α and IL-1β.

Results

Cartilage degradation in 200mg BCE was induced by addition of LR-PRP (33% v/v), demonstrating it as a source of cartilage catabolism. Treatment with proinflammatory cytokines (80ng/ml TNF-α or 8ng/ml IL-1β), ADAMTS-5 (500ng/ml) also resulted in increased sGAG in the medium. Addition of APIC-PRP inhibited cartilage catabolism induced by cytokines, metalloproteinases or LR-PRP in a dose dependent manner.
The addition of LR-PRP at the highest concentration used in the APIC-PRP study reduced but did not inhibit cartilage catabolism induced by cytokines or MMP's measured by the release of sGAG in the medium (data not shown).

Figure 5: APIC-PRP inhibits TNF-α and IL-1β induced cartilage catabolism in a dose dependent manner.



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 that Leukocyte-rich PRP (LR-PRP) contributed to cartilage catabolism, but APIC-PRP protected cartilage from degradation by known OA mediators. This activity can be explained by the 5 – 10 fold increased concentration of alpha-2-macroglobulin (A2M) in APIC-PRP over its concentration in blood. This conclusion is in agreement with our experiments that demonstrate the protective effect of A2M on cartilage. This improved understanding of cartilage biology and metabolism should lead to clinical trials of APIC-PRP in humans.

Disclosure

The authors have Cytonics shares or stock options.
•The APIC-PRP system has not yet been approved by the FDA for human use.

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