

Is There a Chondroprotective Effect of Autologous Protease Inhibitor Concentrate on an Osteoarthritis Rabbit Model? A Pilot Study

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Introduction

The pathology of osteoarthritis involves the upregulation of inflammatory mediators and proteases such as matrix metalloproteases (MMPs). Alpha-2-macroglobulin (A2M) is a naturally-occurring plasma glycoprotein that is a potent protease inhibitor. A2M is believed to modulate cartilage catabolism by its ability to bait, trap and clear MMPs. Though A2M functions throughout multiple tissues and extracellular spaces, it does not normally reach high levels within the intra-articular joint space.

We tested the ability of Cytonic's proprietary Autologous Protease Inhibitor Concentrate (APIC-Cell Free), which concentrates A2M from the blood to inhibit cartilage catabolism, and thereby attenuate the development of osteoarthritis in a ACL-T rabbit model. The rabbit model represents a functional load-bearing *in-vivo* anatomical model for the evaluation of osteoarthritis, which exhibits mechanical properties, morphological structures, and healing capacity similar to human tissues.

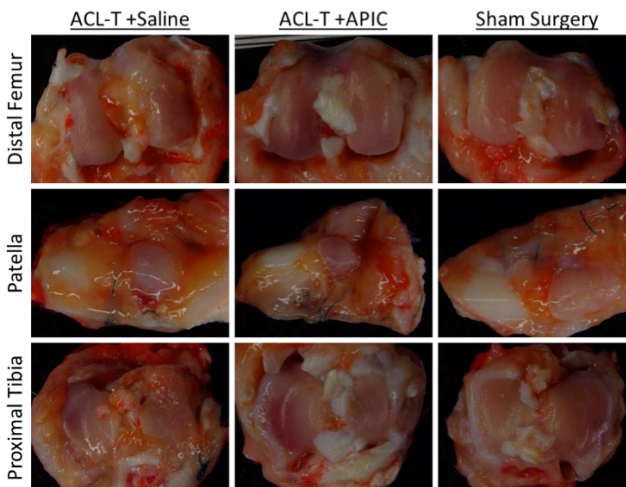
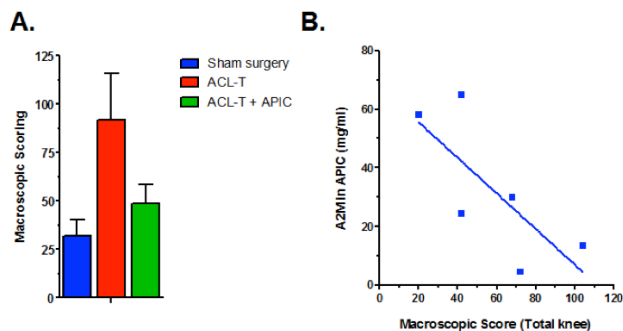


Figure 1
Macroscopic images of rabbit knees 6 weeks after ACL-T and treatment with Saline or APIC Cell Free. Sham surgeries without ACL-T were performed as a control.

Figure 2
Macroscopic evaluation demonstrates an inverse correlation to the concentration of A2M in the APIC Cell Free treatment and cartilage degradation.

Figure 3
Histopathology evaluation of APIC Cell Free-treated rabbit knees demonstrates an inverse correlation between A2M concentration and Safranin-O staining, Structure, Chondrocyte density, and Cluster formation evaluations.

Autologous A2M Concentrate Preparation

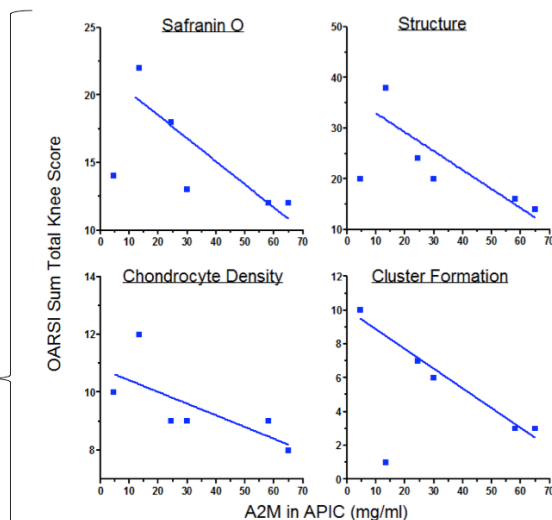
Prior to the initiation of the ACL injury, 20mL of blood was removed from each animal in group 1 and used to prepare the APIC Cell Free concentrate using a series of filters. Every rabbit received the protease inhibitor concentrate from its own blood. Six weeks after the ACL-T operation the animal was sacrificed for macroscopic and microscopic knee joint cartilage evaluation to determine OA progression.

Macroscopic and Histological Analysis

For macroscopic evaluation the distal femoral condyles and tibial plateau surfaces were analyzed and lesions were classified using a validated 0 to 8 scale as previously described. The locations of the lesions in the joint were recorded by a specific nine-area grid of each joint surface, following the classification of the International Cartilage Repair Society (OARSI), which was adapted to the rabbit knee by Lindhorst et al. After macroscopic examination, isolated femoral and tibial samples were fixed and decalcified for histological (microscopic) evaluation.

Results

Macroscopic evaluation of the femur and tibia demonstrated features consistent with cartilage catabolism consistent with OA. Treatment with APIC Cell Free considerably improved cartilage appearance, similar to the sham surgery control (Figure 1). Application of APIC reduced cartilage degradation by $53 \pm 20\%$ compared to untreated controls (mean \pm SEM, $p = 0.0086$) (Fig 2A). The concentration of α -2-Macroglobulin (A2M) in the APIC Cell Free varied from 5 – 65 mg/ml. There was a dose-dependent correlation between higher concentrations of A2M in the APIC Cell Free and decreased OARSI total knee score on the macroscopic evaluation (Figure 2B). There was also a dose-dependent therapeutic benefit to APIC Cell Free treatment observed in sum OARSI histopathology evaluations of Safranin-O staining ($r^2=0.73$), Structure ($r^2=0.76$), Chondrocyte density ($r^2=0.50$), and Cluster Formation ($r^2=0.97$) (Fig 3).



Conclusions

Our data suggest that the Autologous Protease inhibitor Concentrate (APIC-Cell Free), which contains 9 – 10 times the α -2-macroglobulin (A2M) concentration in blood, has a chondroprotective effect on an osteoarthritis rabbit model.

Disclosure

All authors have Cytonics shares or stock options
* The APIC Cell Free system has not been approved by the FDA for human use.

Methods

Female, 8–12 months old, New Zealand white rabbits are used in this study. This rabbit model represents a functional load-bearing *in-vivo* anatomical model for the evaluation of osteoarthritis, which exhibits mechanical properties, morphological structures and healing capacity similar to human tissue.

Multiple Injection Cohort (Group 1): 6 rabbits received ACL-T surgery on the right knee and sham surgery on the left knee. Four injections of 0.3ml Autologous Protease Inhibitor Concentrate (APIC) Cell Free were prepared from the rabbit blood and were administered on day 1, 4, 14, 28 following the ACL knee injury. Rabbits received an equivalent volume of the sterile isotonic saline in the contralateral control knee. The rabbits were monitored for 6 weeks then sacrificed for cartilage degeneration assessment.

Control Group (Group 2): 6 rabbits received ACL-T surgery on the right knee without sham surgery on the left knee. These rabbits were the control group and accordingly did not receive any treatment.