Francois Rannou, MD, PhD
Professor of Medicine
University Paris Descartes, Cochin Hospital, Rehabilitation Department
INSERM U 747, Cartilage Signaling and Pharmacology
Paris, France

Summary of the original abstract

Avocado/Soybean Unsaponifiables Increase Aggrecan Synthesis and Reduce Catabolic and Proinflammatory Mediator Production by Human Osteoarthritic Chondrocytes
YE Henrotin, C Sanchez, MA Deberg, N Piccardi, GB Guillou, P Msika, JYL Reginster
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General Introduction

Osteoarthritis at the chondrocyte level results in decreased matrix production, cartilage extracellular matrix (ECM) degradation, and inflammatory processes. Interleukin-1 (IL-1β) is the key cytokine implicated in these processes. Previous studies have shown a potent protective effect of Avocado Soybean Unsaponifiable (ASU) residues in decreasing cartilage ECM degradation and inflammation and increasing the production of a major actor of the anabolic cascade, transforming growth factor-β (TGF-β). Moreover, the A1S2 quality of ASU residues seems to be the more potent candidate for cartilage protection. However, all the previous *in vitro* studies have been performed under unstable phenotype conditions over time.

Main objective

The main objective of this study is to investigate the effects of A1S2 on human articular chondrocytes cultured with or without IL-1β in a 3-D environment with alginate beads to optimize the phenotype stability of the chondrocytes over long-term culture (12 days).

Methods

Human articular chondrocytes are obtained from the knee joints of adults with primary osteoarthritis immediately after their death. After sequential enzymatic digestion of the cartilage, articular chondrocytes are cultured in alginate beads for 12 days with or without IL-1β (1.7 ng/ml) and A1S2. A1S2 is tested at concentrations from 0.625 to 40.0 µg/ml. In some experiments, ASU residues are also tested at 3.3, 6.6, or 10.0 µg/ml. At the end of the 12-day culture, the culture medium (S), the supernatant from the cell suspension centrifugation (FRM) and the cell pellet with associated matrix (CM) are carefully separated. Cell viability is evaluated by lactate dehydrogenase release in the culture medium. ECM production is evaluated by an aggrecan (AGG) immunoassay. Degradative process is evaluated by matrix metalloproteinase 3 (MMP-3) and tissue inhibitor of MMP-1 (TIMP-1) immunoassays. Inflammatory process is evaluated by prostaglandin-E2 (PGE2), IL-6, IL-8, macrophage inflammatory protein-1 (MIP-1β), and nitric oxide (NO) immuno and Griess assays.

Main results

After 12 days of culture in alginate beads, A1S2 does not alter the chondrocyte viability whatever the concentration used or presence or absence of IL-1β. After 12 days' incubation without IL-1β, the total production of AGG is enhanced by A1S2 in a concentration-dependent manner, with a maximum production for 10 µg/ml. This effect is also observed with A and S added separately, but no additive effect was observed. The increasing production is observed from day 6 to day 12 of culture. When the different compartments (S, FRM, CM) are analyzed individually, the increasing AGG production is seen mainly in the FRM compartment. In the presence of IL-1β, total AGG production decreases greatly, by 73%. Neither A nor S added together or separately are able to reverse this IL-1β catabolic effect. In contrast, A1S2 at 10 µg/ml restores AGG production after 3 days of IL-1β treatment. This recovery is observed after 9 days of A1S2 incubation and reaches 88% production recovery after 15 days of A1S2 incubation. For the ECM degradation process, A1S2 can significantly decrease the IL-1β-induced MMP-3 overproduction from day 3 to day 12 and reverse the inhibitory IL-1β-induced effect on TIMP-1 production. This combined antidegradative effect leads to a significant decrease in ratio of MMP-3 to TIMP-1. For the inflammatory process, A1S2 in the absence of IL-1β dose- and time-dependently inhibits all inflammatory components studied. A1S2 strongly inhibits IL-1β-stimulated PGE2 production. This effect is also observed with separate A and S treatment. However, A1S2 cannot counteract the IL-1β-induced effect on MIP-1β and NO production.

Strengths of the study

One of the strengths of this study is the use of human articular chondrocytes, as compared with previous study, which involved rabbit or calf articular chondrocytes. The other strength is the use of phenotype-stable culture conditions with alginate beads. Under these conditions, A1S2 can counteract the IL-1β-induced catabolic effect by increasing AGG production and can decrease the IL-1β-induced inflammatory effect by decreasing PGE2 production.

Weaknesses of the study

The weaknesses of the study mainly relate to the usual criticisims of *in vitro* experiments. A1S2 pre-treatment before IL-1β treatment would be of interest. The relevance of the ASU-residue doses used is not supported by previous pharmacological results. Finally, transcriptional experiments are lacking.

Conclusion and perspectives

A1S2 at 10 µg/ml can counteract the IL-1β effect by restoring AGG, increasing TIMP-1, inhibiting PGE2, and decreasing MMP-3 production. By these effects, A1S2 is able to restore the balance between the catabolic and anabolic effects. These preliminary results are of interest but need to be enhanced by *in vivo* experiments.