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**Summary of the original abstract**

**Avocado/Soybean Unsaponifiables Prevent the Inhibitory Effect of Osteoarthritic Subchondral Osteoblasts on Aggrecan and Type II Collagen Synthesis by Chondrocytes**
**YE Henrotin, MA Deberg, JM Crielaard, N Piccardi, P Msika, C Sanchez**
**Journal of Rheumatology 2006, 33:1668-1678**

**General Introduction**

Cartilage destruction has been extensively studied to understand the pathophysiological processes involved in osteoarthritis (OA). Recently, the subchondral bone has been found to be another important joint tissue involved in OA. Osteoblasts isolated from subchondral OA bone seem to contribute to abnormal bone remodeling and sclerosis observed in OA. Furthermore, osteoblasts may also contribute to the abnormal remodeling of OA cartilage via microcracks, vascular channels, and neovascularization of the OA subchondral bone. The precise molecular aspects of a putative paracrine phenomenon between subchondral bone and articular cartilage are of particular interest. Using an original co-culture system, Henrotin et al. previously showed that OA subchondral osteoblasts from the sclerotic zone (SC) modulate chondrocyte metabolism by decreasing the mRNA levels of aggrecan, type II collagen (COL2A1), SOX-9, and parathyroid hormone-related protein (PTHrP) in chondrocytes but increasing levels of osteoblast factor 1 (OSF-1), matrix metalloproteinase 3 (MMP-3), and MMP-13, thus leading to a catabolic cartilage process. Because Avocado/Soybean Unsaponifiable (ASU) residues have been shown to modulate chondrocyte metabolism, ASU residues could have a protective effect on the deleterious paracrine relation of osteoblats and chondrocytes observed in OA joints.

**Main objective**

The main objective of this study is to characterize the effect of ASU residues on osteoblast-induced dysregulation of chondrocyte metabolism.

**Methods**

Human articular chondrocytes and osteoblasts are obtained from the knee joints of adults with primary OA immediately after their death. Osteoblasts are from nonsclerotic (NSC) and sclerotic (SC) bone zones. Chondrocytes in alginate beads are co-cultured with monolayer osteoblats for 4 days. In some experiments, osteoblasts are initially cultured for 3 days in the presence of ASU residues. At the end of the culture period, culture medium (S), alginate beads with chondrocytes and monolayer osteoblasts are collected. The alginate beads are dissolved, and the supernatants from cell suspension centrifugation (FRM) and the cell pellet with their associated matrix (CM) are carefully separated. Immunoassays for aggrecan, osteocalcin (OC), interleukin-1β (IL-1β), IL-6, and transforming growth factor-β (TGF-β) are performed directly in the different culture supernatants. An alkaline phosphatase (ALP) assay is performed in the osteoblast cellular fraction. PTHrP is measured in the osteoblast-conditioned culture supernatant. Finally, RNA is extracted from chondrocytes for quantitative real-time RT-PCR for aggrecan, COL2A1, MMP-3, MMP-13, tissue inhibitor of MMP (TIMP-1), TGF-β1, TGF-β3, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2).

**Main results**

The results for SC subchondral osteoblat phenotype markers confirm in basal conditions their altered phenotype as compared to NSC osteoblats by their producing higher amounts of ALP (+ 95%), OC (+ 98%), IL-6 (+ 134%), and TGF-β1 (+ 41%), whereas the amount of PTHrP was decreased (- 35%). In the presence of ASU at 10 µg/ml, the increase in ALP, OC, and TGF-β1 production observed in SC osteoblasts is abolished. When SC osteoblasts are co-cultured with human chondrocytes, the aggrecan content in chondrocyte alginate beads is significantly decreased, by 27% and 31% after 4 or 10 days, respectively, as compared with chondrocyte alginate beads in monoculture. This effect was significantly more marked in the CM than FRM compartment. Furthermore, the decrease in aggrecan content was not observed whereas NSC osteoblasts or normal skin fibroblasts were used instead of SC osteoblasts. Interestingly, in the presence of ASU at 10 µg/ml, the significant decrease in aggrecan content was completely reversed, which suggests a protective role of ASU. These results for aggrecan content are confirmed at the transcriptional level by quantitative real-time RT-PCR. Furthermore, we observe the same response for expression of type II collagen (COL2A1), which suggests a protective role of ASU against the catabolic effect of SC osteoblasts. For the degradative markers (MMP-3, MMP-13) and inflammatory markers (iNOS, COX-2), the increased mRNA level on co-culture with SC osteoblats is not abolished by ASU.

**Strengths of the study**

One of the strengths of this study is the use of human articular chondrocytes from OA patients cultured in stable culture conditions with alginate beads. This is the first study to demonstrate a biological benefit of ASU residues on matrix production by an indirect mechanism implicating SC osteoblats. Furthermore, the experimental procedures to study matrix production investigate the protein and transcript levels and suggest a transcriptional effect. The use of NSC osteoblats and normal skin fibroblasts as a control is of particular interest and strongly supports the importance of the cell phenotype. Finally, because bone is richly vascularized, ASU residues delivered orally may have a systemic effect.

**Weaknesses of the study**

The weaknesses of the study mainly relate to the usual criticisms of *in vitro* experiments. *In vivo* experiments are lacking. Moreover, the precise molecular mechanism is unknown.

**Conclusion and perspectives**

ASU residues can inhibit the catabolic effect of SC osteoblats in OA patients by restoring the cartilage matrix components produced by chondrocytes. These results suggest a potential indirect benefit of ASU via SC osteoblat regulation in addition to their previously well-described direct effect on chondrocytes. These preliminary *in vitro* results are of interest but need to be enhanced by *in vivo* experiments with OA animal models.