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Summary of the original abstract

Stress-induced Signaling Pathways in Hyalin Chondrocytes: Inhibition by Avocado-Soybean Unsaponifiables (ASU)
O Gabay, M Gosset, A Levy, C Salvat, C Sanchez, A Pigenet, A Sautet, C Jacques, F Berenbaum
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General Introduction

Avocado-Soybean Unsaponifiable (ASU) residues have been shown to be able to modulate chondrocyte metabolism at different levels *in vitro*. They decrease the activity of major catabolic and inflammatory factors in human chondrocytes. However, they can act as anabolic factors by increasing the production of transforming growth factor β (TGF-β). Moreover, ASU residues prevent osteoarthritic osteoblast-induced inhibition of the production of matrix molecules, which suggests that these compounds may promote cartilage repair by acting on subchondral bone osteoblasts. By these combined effects, ASU residues could restore the balance between catabolic and anabolic factors. Interleukin 1β (IL-1β) and mechanical stress are the 2 main factors involved in this balance regulation. Molecular signaling of IL-1β and mechanical stress in chondrocytes is mediated by the activation of the transcription nuclear factor κB (NF-κB) and mitogen-activated protein kinases (MAPKs). In the present study, the authors examine whether the observed *in vitro* protective effects of ASU residues could be mediated by inhibition of NF-κB and MAPK pathways in chondrocytes activated by IL-1β or mechanical stress.

Main objective

The main objective of this study is to investigate the effect of ASU residues on IL-1β and mechanical stress signaling pathways in mouse and human osteoarthritic chondrocytes.

Methods

Chondrocytes are obtained from costal, knee, and femoral head cartilage of Swiss mice. Human osteoarthritic articular chondrocytes are obtained during total knee replacement procedures. For the mechanical stress experiments, mouse cartilage explants undergo intermittent compression at 0.5 Hz from baseline for 2 hr. For the cytokine experiments, chondrocytes are incubated with human recombinant IL-1β at 10 ng/ml. For the ASU experiments, chondrocytes or cartilage explants are pre-incubated with 10 µg/ml ASU residues. Prostaglandin E2 (PGE2) production is measured by its release in the chondrocyte culture media by high-sensitivity enzyme immunoassay. Matrix metalloproteinase 3 (MMP-3) and MMP-13 expression is studied by real-time RT-PCR. NF-κB activation is assessed by immunoblotting with an I-κBα antibody, and nuclear translocation of NF-κB is assessed with a p65 antibody. The binding of the p50/p65 complex on DNA is studied by electrophoretic mobility shift assay (EMSA). MAPK activation is assessed with antibodies against phospho- and nonphospho-extracellular signal-regulated kinase 1/2 (ERK1/2) and p38.

Main results

As previously described, ASU residues decrease the IL-1β-stimulated PGE2 release by 55% in treated chondrocytes as compared with non-treated chondrocytes. As well, ASU decrease the IL-1β-induced MMP-3 and MMP-13 expression by 59% and 49%, respectively. In the presence of IL-1β or mechanical stress, ASU decrease the degradation of I-κBα, which suggests an inhibitory effect of ASU residues on the NF-κB signaling pathway. This result is confirmed in human osteoarthritic chondrocytes. Moreover, in the presence of IL-1β, we observe the abolition by ASU of the nuclear translocation of the p65 protein as assessed by immunoblotting. Finally, EMSA results showed that ASU inhibited the p50/p65 complex binding on DNA. For the MAPK signaling pathway, ASU inhibit the IL-1β- and mechanical stress-induced phosphorylation of ERK1/2 but not p38. This result is confirmed in human osteoarthritic chondrocytes.

Strengths of the study

This study uses human articular chondrocytes from osteoarthritis patients in key experiments to suggest a human disease relevance of the results. Moreover, this study is the first to produce *ex vivo* results of ASU biological activity with a 3-D mechanical stress-induced apparatus and perform precise molecular signaling experiments with high-level *in vitro* techniques.

Weaknesses of the study

The weaknesses of the study mainly relate to the usual criticisms of *in vitro* experiments. *In vivo* experiments are needed to confirm these *in vitro* results.

Conclusion and perspectives

ASU residues can interact with the molecular signaling pathways of IL-1β and mechanical stress in mouse and human osteoarthritic chondrocytes, which suggests for the first time the precise molecular effects of ASU residues. These *in vitro* results need to be confirmed by *in vivo* experiments with an osteoarthritis animal model.