Avocado/Soybean Unsaponifiables reverse H_2O_2 decoupling effect on aggrecan, type II collagen and metalloproteases gene expression in human chondrocytes.

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AIM OF THE STUDY.

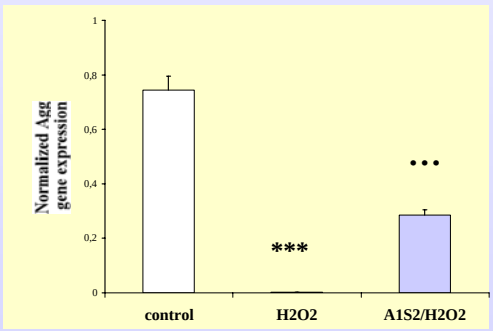
Reactive oxygen species were identified as potent mediators in cartilage tissue destruction and synovial membrane inflammation suggesting their roles in the pathophysiology of joint diseases. Hydrogen peroxide (H_2O_2) is an important second messenger for the regulation of several genes implicated in the degradation of connective tissue. H_2O_2 have been shown to regulate the activation of transcriptional factors like NF-kB and AP-1. These factors activate the expression of a number of genes (metalloproteases, interleukin-1ß,…) involved in the pathogenesis of osteoarthritis (OA). This study was designed to investigate the action of sublethal doses of H_2O_2 on cartilage matrix components (aggrecan (Agg), type II collagen (Col II)) and matrix metalloproteases (MMP-3, MMP-13) gene expression. The effects of Avocado/Soybean Unsaponifiables (ASU), a symptomatic acting drug in osteoarthritis (OA), were also investigated.

METHODS.

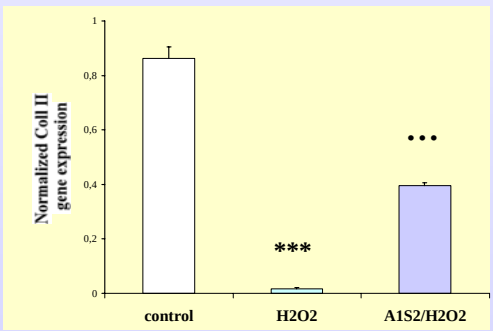
Human chondrocytes were enzymatically isolated from OA knee cartilage. Chondrocytes were cultured for 24 h in a well-defined culture medium in the presence or not of ASU mixed in a ratio 1:2 (A1S2, 1 part of avocado and 2 parts of soybean, 10 µg/ml). After washings, the cells were incubated with H_2O_2 (10^{-4} M) for 3 h in HBSS, and thereafter, cultured in a well-defined culture medium for the next 24 h. Agg, Col II, MMP-3 and MMP-13 gene expressions were quantified by reverse transcription of mRNA followed by real time and quantitative polymerase chain reaction (RT PCR, LightCycler, Roche).

RESULTS.

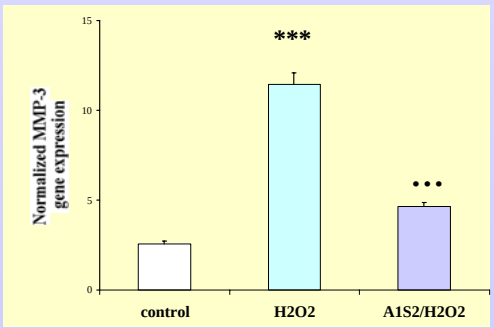
H_2O_2 markedly decreased Agg and Col II mRNA levels but increased those of MMP-3 and MMP-13 (***, $p < 0.001$). Pre-incubation with A1S2 significantly counteracted the H_2O_2 effects on Agg, Col II and MMP-3 gene expressions (•••$p < 0.001$). No significant effect was observed for MMP-13 gene expression.



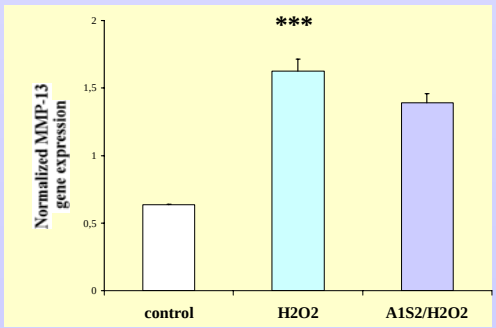
Effect of A1S2 on Agg gene expression



Effect of A1S2 on Col II gene expression



Effect of A1S2 on MMP-3 gene expression



Effect of A1S2 on MMP-13 gene expression

CONCLUSIONS.

- **Sublethal H_2O_2 concentrations have catabolic actions on cartilage by :**
  - **Decreasing Agg and Col II gene expressions**
  - **Increasing MMP-3 and -13 gene expressions.**
- **These deleterious effects of H_2O_2 can be prevented by ASU, giving a new rationale explanation to the potential structural effect of this drug in OA.**