AVOCADO/SOYBEAN UNSAPONIFIABLES INCREASE AGGRECAN SYNTHESIS AND REDUCE CATABOLIC AND PRO-INFLAMMATORY MEDIATORS PRODUCTION BY HUMAN OSTEOARTHRITIC CHONDROCYTES

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AIM OF STUDY : To investigate the long-term effects (12 days) of avocado/soybean unsaponifiables (ASU) on the metabolism of human osteoarthritic (OA) chondrocytes cultured in alginate beads.

METHODS : Enzymatically isolated OA chondrocytes were cultured in alginate beads in a well-defined culture medium for 12 days, in the presence or not of 10^{-10} M IL-1β. The DNA content was measured according to a fluorimetric method. Aggrecan (AGG), stromelysin-1 (MMP-3), tissue inhibitor of metalloproteinases-1 (TIMP-1), macrophage inflammatory protein-1 beta (MIP-1β), interleukin (IL)-6, -8 and TGF-β1 production were assayed by specific enzyme amplified sensitivity immunoassays (EASIA). Prostaglandin (PG) E_2 was measured by a specific radioimmunoassay and nitrite by a spectrophotometric method based upon the Griess reaction. Avocado and soybean unsaponifiable residues were mixed in the ratio 1:2 (A_1S_2) or tested separately in a range of concentrations between 0.625 and 40 µg/ml.

Figure 1: Effect of A1S2 10 µg/ml on AGG synthesis recovery after a 3-day treatment with IL-1β 10^{-10} M. The curves represent the total production, which corresponds to the sum of AGG found in S, CM and FRM. In this set of experiments, chondrocyte cultures were pre-treated or not (dotted line, - - - -) for 3 days with 10^{-10} M IL-1β , washed and then cultured for the next 15 days (between day 0 to day 15) in the absence (-■-) or in the presence (- -) of A1S2 10 µg/ml. Values are means SEM (n= 9) of three independent cultures performed with cartilage specimens coming from three different donors. Statistical significance in comparison to the controls (treated by IL-1β): *** p<0.001.

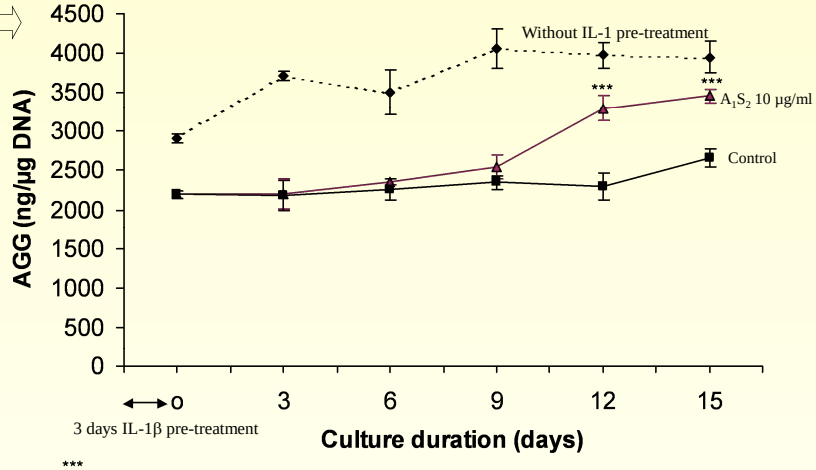
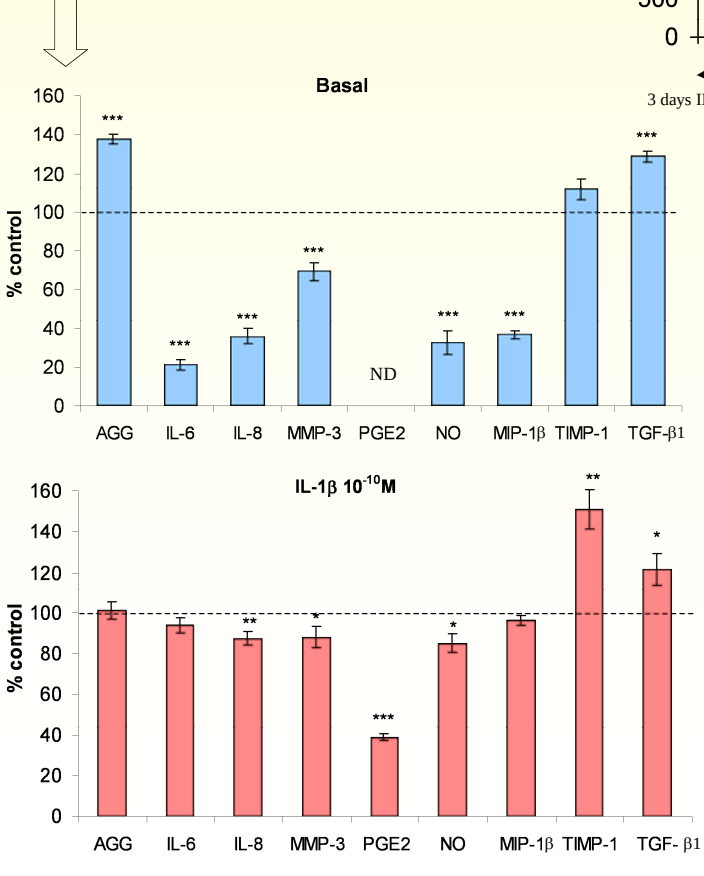


Figure 2 : Global effects of A1S2 10 µg/ml after a 12 days culture period, in the absence or presence of 10^{-10} M IL-1β. Results, represented as percentage of the control values for each parameter, are means SEM (n= 9) of three independent cultures performed with cartilage specimens coming from three different donors. * p<0.05, ** p<0.01 and *** p<0.001



RESULTS: After twelve days of incubation, A_1S_2 increased in a dose- and time-dependent manner AGG synthesis and accumulation in alginate beads. Furthermore, A_1S_2 promoted the recovery of aggrecan synthesis after a three days period of IL-1β treatment (figure 1). ASU were potent inhibitors of both basal and IL-1β-stimulated MMP-3 productions, and stimulators of TGF-β1 synthesis. They also partially reversed the inhibitory effect of IL-1β on TIMP-1 production (figure 2). Furthermore, ASU inhibited MIP-1β, IL-6, IL-8, ·NO and PGE_2 basal production by OA chondrocytes and partially counteracted the IL-1-stimulating effect on PGE_2. In A_1S_2 mixture, A and S had an additive effect on AGG synthesis and acted in synergy to inhibit IL-8 and ·NO production (data not shown).

CONCLUSIONS : From this work, we can conclude that ASU stimulated aggrecan production and recovered aggrecan production after IL-1β treatment, probably through the production of TGF-β1. In parallel, ASU decreased MMP-3 production and stimulated TIMP-1 production. Taken together, these findings give an explanation to the recently demonstrated structure-modifying effects of ASU in hip osteoarthritis (1).

(1) M Lequesne, E Maheu, C Cadet, RL Dreiser. Structural effect of avocado/soya unsaponifiables (ASU) on joint space loss in osteoarthritis of the hip. Arthritis Rheum 2002;47:50-58.