ENHANCEMENT OF NSAIDS ANTI-INFLAMMATORY EFFECT BY AVOCADO/SOYBEAN UNSAPONIFIABLES, GLUCOSAMINE, CHONDROITIN SULFATE COMBINATION IN IL-1β ACTIVATED CHONDROCYTES

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INTRODUCTION:
Joint inflammation involving articular cartilage, synovium, and subchondral bone plays a critical role in the pathogenesis of osteoarthritis (OA). Pain and cartilage breakdown that characterize OA are attributed to excess production of mediators including prostaglandin E2 (PGE2), cytokines, chemokines, nitric oxide, and reactive oxygen species (1). Chondrocytes and other tissue cells in the joint synthesize these inflammatory molecules. Non-steroidal anti-inflammatory drugs (NSAIDs) and COX-2 selective drugs are used extensively to ameliorate inflammation and pain in OA. However, life threatening side effects caused by these agents have prompted the search for alternative or complementary strategies. Avocado/soybean unsaponifiables (ASU), glucosamine (GLU), and chondroitin sulfate (CS) individually or in combination are reported to reduce inflammation. These are reported to be beneficial for the management of OA (2-4). The present study determined whether sub-therapeutic, reduced concentrations of NSAIDs in combination with [ASU+GLU+CS] will suppress the inflammatory response more effectively than either treatment alone. We used a canine chondrocyte microcarrier spinner culture model to evaluate inhibition of cytokine induced PGE2 production by these agents. The dynamic microcarrier culture system facilitates chondrocyte proliferation while maintaining their phenotype (5). Like humans, canines suffer from OA and similar NSAIDS are being used for treatment.

METHODS:
Canine chondrocytes from articular cartilage were propagated in microcarrier spinner culture and analyzed by H&E staining, SEM, and TEM. Phenotype was evaluated by immunofluorescent staining for type II, type I collagen, and aggrecan. To evaluate inflammatory response, the following preparations were added to 10 ml of culture aliquots: (1) control media; (2) IL-1β (R&D Systems, 50ng/mL); (3) meloxicam (Sigma, 11.5ng/mL) + IL-1β; (4) [ASU (NXM-1000TM, 25 μg/mL) + GLU (FCHG9®, 15μg/mL) + CS (TRH122®, 20μg/mL)] + IL-1β, (5) carprofen (Pfizer, 40 ng/mL) + IL-1β, (6) meloxicam + [ASU+GLU+CS] + IL-1β, or (7) carprofen + [ASU+GLU+CS] + IL-1β. Supernatant from cultures following a 24 hr incubation at 37°C, 5% CO2 was assayed for PGE2 by ELISA. Pair-wise multiple comparisons were performed using one-way ANOVA, Tukey post-hoc with SigmaStat statistical software (Windows Version 3.11) where P<0.05 was considered statistically significant.

RESULTS:
Chondrocytes proliferated and produced extracellular matrix on microcarriers (Figure 1 A, B). Cell-seeded microcarriers formed aggregates by seven days (Figure 1A, B). Chondrocytes immuno-stained intensely for type II collagen and aggrecan (Figure 2) while type I aggregates by seven days (Figure 1A, B). Chondrocytes immuno-stained intensely for type II collagen and aggrecan (Figure 2) while type I aggregates by seven days (Figure 1A, B). Chondrocytes immuno-stained intensely for type II collagen and aggrecan (Figure 2) while type I aggregates by seven days (Figure 1A, B). Chondrocytes immuno-stained intensely for type II collagen and aggrecan (Figure 2) while type I aggregates by seven days (Figure 1A, B).

DISCUSSION:
The key finding in this study is that sub-therapeutic concentrations of NSAIDs combined with [ASU+GLU+CS] more effectively inhibited the inflammatory pathway than the individual treatment alone. This observation suggests the feasibility of reducing NSAID dosing to minimize adverse effects while effectively attenuating inflammation by combining with [ASU+GLU+CS].

REFERENCES:

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