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OLEUROPEIN, QUERCETIN AND CURCUMIN PREVENT CARTILAGE BREAKDOWN IN BOVINE CHONDROCYTES

F. Membrez Scalfo †, S. Escande †, S. Schulsse †, E.A. Offord †, Y. Henrotin ‡, M-N. Horcajada †. † *Nestlé Res. Ctr., Lausanne 26, Switzerland;* ‡ *Bone and Cartilage Res. Unit, Liège, Belgium*

Purpose: Although osteoarthritis has long been considered as a wear and tear disease leading to loss of cartilage, cytokines such as interleukin 1 and tumor necrosis factor alpha produced by activated chondrocytes and synoviocytes act a major role in the catabolic process. The aim of this study was to investigate the influence of natural anti-inflammatory and antioxidant polyphenols from olive (oleuropein) or fruit and vegetables (rutin or curcumin), at physiological doses, on metabolic functions of chondrocytes in the presence or absence of an inflammatory stimulation which mimics OA inflammation and disease progression.
Methods: Chondrocytes were isolated from bovine cartilage and cultured for 3 or 12 days in alginate beads with or without oleuropein (1.5μM), quercetin (1.5μM), curcumin (1.5μM) or combination of them by two or three (1.5μM of each polyphenol) in presence or absence of an inflammatory stimulation (IL-1α; 10 ng/ml). The transcription levels of several extracellular matrix components (Type-II collagen, aggrecan), pro-inflammatory genes (iNOS, COX-2) and catabolic enzymes (MMP-3, MMP-13, and ADAMTS-5) were measured in mRNA after 3 days of culture. Extracellular matrix components such as sulfated-glycosaminoglycan, aggrecan and collagen were measured after 12 days of culture by biochemical analyses and normalized by DNA content. Cytotoxicity of these phytonutrients at physiological doses was analyzed by LDH release.
Results: No significant difference in cytotoxicity after 12 days was observed with the addition of oleuropein, quercetin and curcumin alone or in various combinations. When stimulated with IL-1α, oleuropein, quercetin and curcumin treatments, alone or in combination significantly decreased collagenases (MMP-3 and MMP-13) and aggrecanase (ADAMTS-5) gene expression. Collagen release in the media was only decreased by combination of the three polyphenols (4.5μM total). Aggrecan production was increased by quercetin and curcumin alone. Besides, combination of oleuropein with quercetin or curcumin (3.0μM total) significantly increased type-II collagen mRNA levels. However only oleuropein combined with curcumin significantly increased aggrecan gene expression and sulfated-glycosaminoglycan in supernatant after 12 days. Regarding inflammatory mediators, iNOS gene expression was only modulated by combination of the three polyphenols. Furthermore, COX-2 mRNA levels tended to be inhibited in a dose-dependent manner whatever the treatments.
Conclusions: We have demonstrated that, in our experimental conditions, oleuropein, quercetin and curcumin could limit inflammatory and catabolic mediators secreted by chondrocytes, suggesting they may prevent cartilage degradation. However, even combination of oleuropein with quercetin or curcumin were both effective on cartilage breakdown, extracellular matrix synthesis was mainly enhanced by exposure to oleuropein with curcumin. In conclusion, all compounds showed efficacy to reverse the imbalance between anabolic and catabolic factors in chondrocytes, suggesting that these dietary polyphenols could be efficient in the management of osteoarthritis.

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A MODIFIED AND ENHANCED ATDC5 CHONDROGENESIS MODEL PRODUCES AN ARTICULAR-LIKE PHENOTYPE

M. Mayhew †, L. Kevorkian ‡, T. Swingler †, D. Bevan †, C. Stubberfield ‡, A. Moore ‡, J. Gavrilović †. † *Univ. of East Anglia, Norwich, United Kingdom;* ‡ *UCB Pharma Ltd, Slough, United Kingdom*

Purpose: Differentiating mesenchymal stem cells are often used as an alternative supply of chondrocytes that allow observations not only into the molecular signalling events that occur during chondrogenesis, but also in mature cartilage tissue. The resulting tissue can then also be used to model various cartilage pathologies or to attempt cartilage repair. It is difficult to generate the required phenotype of the chondrocytes/tissue produced and models may therefore not accurately reflect the appropriate type of cartilage tissue required, which has different functions and properties depending on the location within the body. Since osteoarthritis is a disease that degrades articular cartilage, the purpose of this study was to identify a culturing model with physiological relevance that specifically produces articular cartilage-like tissue.
Methods: ATDC5 chondrogenic precursor cells were grown in three-dimensional, high-density micromass, stimulated with a combination of insulin (which induces differentiation in ATDC5 cells) and ascorbic acid over time, and directly compared to traditional ATDC5 differentiation in monolayer by insulin stimulation alone. Quantitative (q)RT-PCR was used to identify regulation of various genes related to chondrogenesis, chondrocyte maturation/hypertrophy (reminiscent of growth plate cartilage) and markers of the articular cartilage phenotype. Confirmation of some genes was observed at the protein level by immunofluorescence and histological staining.
Results: ATDC5 cells induced to differentiate in the traditional monolayer culture developed heterogeneously, forming aggregates termed "cartilage nodules", which stained for Alcian blue and increased in number with ascorbic acid stimulation. Areas between nodules did not stain with Alcian blue, indicating a heterogeneous mixture of chondrogenic and non-chondrogenic areas, confirmed by Type II Collagen immunofluorescence. In contrast, central micromasses remained as one large nodule, with noticeably extensive extra-cellular matrix deposition, staining deeply and uniformly with Alcian blue, indicating more homogenous differentiation. By qRT-PCR, differentiating micromasses stimulated with ascorbic acid for three weeks had a 231-fold increase in *Col2a1* expression (Type II Collagen) compared to traditional differentiating monolayer cultures with stimulation by insulin alone. *Frzb*, a secreted Wnt antagonist previously described as a marker for articular cartilage, was increased 35-fold in the same comparison. *Col10a1* (Type X Collagen), a hypertrophic region marker, was increased with micromass culture, but protein was immunolocalised to a small region within the central micromasses surrounded by extensive Type II Collagen staining. Other markers of hypertrophic differentiation, which would resemble a more growth plate-like phenotype, were either not expressed (*Mmp13*) or did not demonstrate altered expression (*Runx2*). Taken together, these results suggest that a marked difference in gene expression is seen when ATDC5 cells are cultured in micromass with ascorbic acid.
Conclusions: Using the traditional culturing method in monolayer with insulin stimulation alone, ATDC5 chondrogenesis occurs heterogeneously, forming separate cartilage nodules, which we showed can be increased in number with ascorbic acid stimulation. Using a three-dimensional culturing technique, which is more physiologically relevant, and combining this with ascorbic acid stimulation, we showed that chondrogenesis was both more homogenous in nature and also highly enhanced expression of markers of chondrogenesis and of articular cartilage. We therefore suggest that this modified culture system for ATDC5 cells provides a good model for studying articular cartilage.

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PIT1/SLC20A1 REVEALED AS A KEY PLAYER OF THE CHONDROCYTE SURVIVAL

G. Couasnay. *INSERM, Nantes, France*

Purpose: PiT1/Slc20a1 has been largely described as a plasma membrane protein functioning as a high affinity Na+-phosphate (Pi) cotransporter. During the development of long bones, PiT1 is mainly expressed in hypertrophic chondrocytes. With respect to this localization and to the importance of Pi in bone biology, many in vitro studies have suggested that PiT1 could represent the main supplier of Pi needs during mineralization. Complete deletion of PiT1 in mice resulted in embryonic lethality at E12.5, preventing the analysis of its implication in bone physiology. Recently, we have generated and analyzed hypomorphic mice expressing 15% of PiT1, a level that was enough to allow survival of the mutant animals. These mice presented with a bone mineralization delay during the first fifteen days of life which was rapidly compensated with age. Interestingly, this phenotype arose despite a normal Pi uptake most likely due to a functional compensation by PiT2, the paralog of PiT1. Of importance, the PiT1 hypomorphic mice displayed an anemia, which could interfere with the interpretation of the data.
Methods: To avoid confounding effects of this animal model, we have generated an inducible chondrocyte-specific PiT1 knockout mice model