Purpose: Rheumatoid arthritis (RA) synoviocytes present an increased mitochondrial genome mutagenesis, leading to mitochondrial alterations that might be participating in the pathogenesis of the disease. This study was performed to examine whether mitochondrial dysfunction is involved in joint destruction and inflammation in RA in relation to the expression of matrix metalloproteinases-1 and -3 (MMP-1 and MMP-3) and vascular endothelial growth factor (VEGF) in normal human synoviocytes.
Methods: Normal human synoviocytes were treated with the commonly used mitochondrial respiratory inhibitor oligomycin (OLI) (10 μg/ml) for 24 hours. As a positive control, MMPs and VEGF expression was induced by 5ng/ml interleukin-1β (IL-1β). MMPs and VEGF mRNA expression (RT-PCR) and MMP-1 protein levels (ELISA) were analyzed. The effect of the natural antioxidant resveratrol (RSV, 50 μM) was tested.
Results: We found that oligomycin-induced mitochondrial dysfunction in synoviocytes for 24h significantly increased MMP-1 mRNA expression (835,7±480,4 vs. Basal 1, n=6, $p<0.05$), even more than higher levels of the positive control IL-1β (580,3±343,2). This increase in MMP-1 mRNA expression was accompanied by the synthesis of MMP-1 protein (7020±2187 vs. Basal 2780±856 pg/250.000 cells, n=6, $p < 0.05$). When MMP-3 and VEGF were evaluated the response was also increased. Besides, mitochondrial dysfunction synergizes with the cytokine IL-1β (1ng/ml) to induce the expression of the destructive mediators MMP-1 and-3. We found that the co-treatment of synoviocytes with OLI for 24h significantly increased the MMP-1 and-3 mRNA expression induced by IL-1β (3249,8% and 331,4% vs. IL-1β 100%, respectively). Similar results were observed in MMP-1 protein expression. Finally, the natural antioxidant RSV showed a significantly reduction in the destructive response attenuating this effect at mRNA (8794±4423 IL-1β+OLI vs. 2069±1500 RSV+IL-1β+OLI) and protein (53474±14784 pg/250.000 cells IL-1β+OLI vs. 5856±1456 RSV+IL-1β+OLI) expression.
Conclusions: Mitochondrial dysfunction may play a significant role in the pathogenesis of RA by stimulating the production of MMPs and VEGF in synovial cells, leading to joint destruction and inflammation, respectively. The natural antioxidant RSV also proved to significantly reduce this destructive response.

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INHIBITION OF HYDROGEN PEROXIDE, LIPOPOLYSACCHARIDE AND INTERLEUKIN-1 BETA-INDUCED PROSTAGLANDIN E-2 PRODUCTION IN CHONDROCYTES BY THE COMBINATION OF AVOCADO/SOYBEAN UNSAPONIFIABLES AND LIPOIC ACID

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Purpose: The present study determined whether prostaglandin E2 (PGE_2) production induced by hydrogen peroxide (H_2O_2), lipopolysaccharide (LPS), or interleukin-1β (IL-1β) can be inhibited by the combination of avocado/soybean unsaponifiables (ASU) and lipoic acid (LA). ASU is documented as an anti-inflammatory agent whereas LA has both anti-inflammatory and antioxidant properties.
Inflammation and oxidative stress have been proposed to play a role in the pathogenesis of osteoarthritis (OA). These two processes are thought to be coupled as suggested by the observation that generation of reactive oxygen species (ROS) can be induced in articular chondrocytes by inflammatory mediators including cytokines and prostaglandins. Conversely, ROS such as H_2O_2 can also induce production of inflammatory mediators. During oxidative stress, elevated concentrations of ROS inflict damage to DNA, proteins, and subcellular structures resulting in chondrocyte death by apoptosis. ROS also inhibit proteoglycan synthesis and facilitates cartilage degradation. In comparison, inflammatory mediators such as PGE_2 induce pain and cartilage breakdown.
Methods: Equine chondrocytes were pre-incubated at 37°C, 5% CO_2 with: (i) control media alone, (ii) ASU (NMX1000®, 8.3 μg/ml) and LA (1.25 or 2.5 μg/ml), (iii) ASU (NMX1000®, 8.3 μg/ml), or (iv) LA (1.25 or 2.5 μg/ml), for 24 hrs. Chondrocytes were then exposed to H_2O_2 (500 μM), LPS (1 ng/ml), or IL-1β (10 ng/ml) for another 24 hrs. Production of PGE_2 was measured by ELISA. NF-κB translocation was assessed by Western blot. Data was analyzed using one-way ANOVA, Tukey post-hoc at $P<0.05$ level of significance.
Results: PGE_2 production in chondrocyte cultures significantly increased following exposure to H_2O_2 (1.5-fold) or LPS (3-fold). Pre-incubation of chondrocytes with the combination of ASU+LA (1.25 or 2.5 μg/ml) before exposure to either H_2O_2 or LPS, significantly inhibited PGE_2 more than either ASU or LA alone ($P<0.05$). In comparison, exposure to IL-1β increased PGE_2 production by 2000-fold which was significantly inhibited by the combination of ASU+LA (1.25 or 2.5 μg/ml, $P<0.05$). Inhibition of cytokine-induced PGE_2 production was associated with inhibition of NF-κB nuclear translocation.
Conclusions: This study demonstrates that H_2O_2, LPS, or cytokine-induced PGE_2 production in chondrocytes can be significantly inhibited by the combination of ASU+LA. The effect involves down-regulation of NF-κB, a key transcriptional regulator of inflammation. This finding suggests that the combination of ASU+LA affects multiple targets and may be beneficial for the management of inflammation in the joint and associated conditions of oxidative stress.

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EFFECTS OF BRAZILIN ON HUMAN CHONDROCYTES AND SYNOVIOCYTES

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Purpose: Osteoarthritis (OA) is the most prevalent disorder of the musculoskeletal system and has a very high socioeconomic impact. Current treatment options for OA are only symptomatic and do not act on the causes of OA or stop the progression of the disease. Sappan Lignum, the heartwood of Caesalpinia sappan L. (Leguminosae), has been widely used in oriental traditional medicine for improvement of blood circulation, sprains, and emmeniopathy. Different extracts and active compounds such as brazilin have been reported to possess antioxidative and anti-inflammatory effects. This study investigates the influence of brazilin on the expression of IL1ß, TNF-alpha, PGE-2, MMP3 and MMP13 in primary chondrocytes and synoviocytes of OA patients.
Methods: Brazilin was isolated from Caesalpina sappan using preparative HPLC and identified using 1H-NMR. Chondrocytes and synoviocytes were isolated from OA patients undergoing joint replacement. The cytotoxicity of Brazilin was assessed using an MTT-based test. Cells were preincubated with Brazilin (5, 10, 20 μg/ml) for 1 h and then incubated for 6 h in the presence of 10 ng/ml IL-1ß prior to RNA isolation and qPCR analyses of target gene mRNA levels. Target protein concentrations in the cell culture supernatants were measured using ELISA.
Results: There was no significant cytotoxicity of brazilin up to concentrations of 40 μg/ml in primary chondrocytes and synoviocytes. COX-2, TNF-alpha and MMP3 mRNA levels in primary chondrocytes were strongly increased up to 200 fold by 10 ng/ml IL-1ß, whereas pre-treatment with 5-20 μg/ml brazilin significantly reduced this effect dose dependently. In agreement, pretreatment with 10 μg/ml brazilin significantly suppressed the IL-1ß-induced TNF-alpha and MMP3 production in cell culture supernatants. In IL1ß stimulated primary synoviocytes, Brazilin markedly suppressed IL1ß, TNF-alpha and MMP13 mRNA and protein levels.
Conclusions: In summary, we isolated brazilin, identified its structure, and determined its biological activities on OA chondrocytes and synoviocytes. Brazilin substantially suppressed the expression of proinflammatory mediators and major MMPs suggesting that brazilin may be beneficial for future management of OA.

Mechanobiology

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THE PRIMARY CILIUM ORCHESTRATES CHONDROCYTE MECHANOTRANSDUCTION

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Purpose: To understand the role of the chondrocyte primary cilium in mechanotransduction. The primary cilium is a singular organelle protruding outward from the mature centriole into the extracellular space, decorated with many receptors and integrins. The tubulin scaffold, ciliary membrane and proteome are constructed and maintained by dynein motors and intraflagellar transport cargos. In many cell types such as kidney epithelia, vascular endothelium and osteocytes, primary cilia are involved in mechanotransduction, often through the activities of polycystin 1 and 2 located on the cilium. Physiologically cartilage experiences mechanical signals, altering cell behaviour, that include compression and fluid flow. It is thought aberrant mechanotransduction may be one factor in the development of tissue pathology. Here we test the hypothesis that the cilium is essential for chondrocyte mechanotransduction and the up-regulation of proteoglycan synthesis through an established purinergic pathway involving the release of ATP and subsequent activation of Ca2+ signalling.
Methods: To test this we used a hypomorphic mutation of Tg737, which encodes for IFT88, and abolishes genesis and growth of the cilium and which has been shown in vivo to result in murine matrix patterning defects. A well-established 3D agarose culture system was implemented to allow compressive loading of murine WT and Tg737 chondrocytes in culture followed by the quantification of ATP release with a luciferase assay, calcium transients by means of Fluo-4 imaging, and matrix production by qPCR and biochemical assay. Additionally expression of purinergic receptors (P2R) and polycystins (PC) 1 and 2 were assessed by western blot and immunocytochemistry.
Results: Compression of WT chondrocytes increased calcium transients ($p<0.05$) and matrix production at gene and protein levels ($p<0.05$) however these mechanosensitive responses were not present in Tg737 chondrocytes. Mechanosensitive ATP release ($p<0.01$) was maintained between WT and Tg737 cells implying that the cilium is required for ATP reception or transduction. Indeed, exogenous addition of ATP up-regulated Ca2+ transients in WT ($p<0.001$) but did not in Tg737 cells, although there were no differences in P2R expression. In Tg737 cells PC-1 expression was altered such that the full size protein product was absent.
Conclusions: We conclude that the primary cilium is essential for chondrocyte mechanotransduction through the regulation of purinergic Ca2+ signalling. We speculate that this may be attributed to a role for the cilia protein polycystin-1. This demonstrates the central role for the chondrocyte primary cilium in cartilage physiology in the context of the chondrocyte response to mechanical stimuli.

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MECHANICAL STRAIN STIMULATES HEDGEHOG SIGNALLING IN ADULT ARTICULAR CHONDROCYTES AND REDUCES PRIMARY CILIA LENGTH

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Purpose: Hedgehog proteins constitute a family of secreted morphogens that have crucial roles in skeletal development. Indian Hedgehog (Ihh), a member of this family, regulates chondrocyte proliferation and differentiation and directs endochondral bone formation. During development Ihh exhibits mechanosensitive gene expression and is proposed to act as a transducer of mechanical signals. Hedgehog signalling is aberrantly activated in osteoarthritis and promotes cartilage degradation through the upregulation of ADAMTS-5. However, the mechanism responsible for up-regulating hedgehog signalling is unknown. Hedgehog signalling requires the primary cilium, a microtubule based organelle that projects out from the cell into the extracellular space.
We propose that mechanical loading of adult chondrocytes regulates Ihh gene expression and influences primary cilia structure leading to complex, dose dependent mechanoregulation of hedgehog signalling and downstream ADAMTS-5 activity.
Methods: Articular chondrocytes were harvested from adult bovine metacarpal-phalangeal joints. Chondrocytes were subjected to 5, 10 or 20% cyclic tensile strain at a frequency of 0.33Hz for 1hr. Quantitative real-time PCR was used to monitor the gene expression of Ihh, ADAMTS-5 and Patched-1 (Ptch1). Changes in the expression of Ptch1 were used as a measure of Hh pathway activation. Primary cilia prevalence and length were quantified using confocal images of cells labelled with acetylated α-tubulin ($n>300$ cells).
Results: Ihh gene expression was significantly upregulated at all strain levels ($p<0.05$). No significant differences were observed between strain magnitudes. Ptch1 gene expression was significantly increased at 5% and 10% strain ($p<0.05$) indicative of hedgehog pathway activity. This was accompanied by significant increases in ADAMTS-5 gene expression ($P<0.05$). No significant changes in Ptch1 or ADAMTS-5 expression were observed at 20% strain. Mean primary cilia length was significantly reduced by mechanical strain ($p<0.05$) in a dose-dependent manner with cilia becoming progressively shorter with increasing strain magnitude. No change in primary cilia prevalence was observed.
Conclusions: We have shown for the first time that Ihh exhibits mechanosensitive expression in adult articular chondrocytes. We demonstrate the mechanonsensitive activation of hedgehog signalling and upregulation of ADAMTS-5 expression. In addition we show changes in chondrocyte primary cilia length may lead to the attenuation of hedgehog signalling. Greater cilia length, as reported in osteoarthritis, is associated with greater hedgehog-mediated ADAMTS-5 expression and will result in greater cartilage degradation. Thus these two mechanisms may explain the aberrant upregulation of hedgehog signalling in osteoarthritis.

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EX VIVO MECHANICAL STIMULATION COUNTERACTS IL-1 EFFECT ON HUMAN OA CARTILAGE EXPLANTS

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Purpose: Mechanical loading regulates development and long-term maintenance of joint tissues that are sensitive to the magnitude, duration and nature of mechanical stimuli. Accumulating evidence suggests that physiological joint loading helps maintain cartilage integrity while reduced loading and overloading have catabolic effects. Mechanical stimulation is also involved in the pathogenesis of osteoarthritis. However, the mechanisms of regulation of joint homeostasis by mechanical stimuli are not completely elucidated. In particular, most of the studies on mechanical stimulation has been conducted on isolated chondrocytes, while few data are available on human cartilage tissue. We aimed to investigate chondrocyte response induced by ex vivo compression of human OA cartilage explants in basal conditions and in the presence of pro-inflammatory stimuli.
Methods: Samples were collected from ten OA patients, aged 72,2 ± 7,02 (mean ± SD) years, undergoing knee replacement surgery. For each specimen at least 16 full thickness biopsies were obtained and subjected to controlled physiological compression in the Flexcell FX-4000C stage presser apparatus (Flexcell International Corporation, USA): three rounds of stimulation of 4hrs with 20 hrs interval, 6% compression (36 kPa), 1Hz frequency. After mechanical stimulation, biopsies were recovered and used for total RNA extraction (pulverization at MicKrodismembrator S, Sartorius, Italy) and /or stored for subsequent histological and immunohistochemical analyses. Semi-quantitative RT-PCR analysis of aggrecan, collagen II, SOX9, aggrecanases ADAMTS 4 and 5 was performed in respect to GAPDH expression. Culture supernatants were analysed for sulfated glycosaminoglycan (GAG) content by Dimethylmethylene blue assay. Mechanically stimulated samples were compared to non-compressed ones and also evaluated under pro-inflammatory stimuli: IL-1β (2 ng/ml) alone or in combination with IL-4 (10 ng/ml), selected for its pivotal role in chondrocyte anabolic response to mechanical stimulation.
Results: Despite the very low amount of available cartilage tissue from human OA biopsies it was possible to quantify different cartilage matrix markers of homeostasis from the recovered total RNA. Relative mRNA expression indicated that mechanical stimulation modify the expression of aggrecan, collagen II, sox9 and ADAMTS4 molecules. In particular, we observed that the proinflammatory effect of IL-1 on collagen II and aggrecan was significantly counterbalanced by mechanical compression both in basal conditions and in the presence of IL-4 (collagen II: $p=0.018$ basal vs. IL-1 and $p=0.043$ IL4 vs. IL-1 in non compressed samples, not significant after compression; aggrecan: $p= 0.018$ basal vs. IL-1 and