used in experiments up to the 2nd passage. Two experimental protocols were used: a membrane water permeability assay and measurement of intracellular calcium using Fura-2AM.
Results: Using an established permeability assay, whereby a hypotonic solution is applied to the cells and cell volume recorded, aquaporin permeability was measured. In healthy cells permeability was found to be 31±3x104cm.s (n = 5). Stimulated cells did not show a significant change in aquaporin permeability (n = 12). Calcium measurements showed that healthy cells responded to the same hypotonic challenge with an intracellular calcium increase of 115±15nM (n = 16). This calcium increase was inhibited by the TRP channel antagonist, PYR3 (n = 69). When applied to cells from the in vitro model of arthritis the same osmotic challenge caused a significantly greater calcium increase of 328±45nM (n = 11; p≤0.01).
Conclusions: We have investigated changes to two ion channels in healthy chondrocytes and those from an in vitro model of arthritis. Aquaporin function appeared unchanged in our cytokine arthritis model, possibly suggesting that this change in gene expression occurs as a result of OA, rather than contributing to OA development. Previous work has shown that intracellular calcium increases greater than :300nM can lead to cell apoptosis and therefore the changes we observe here could implicate important pathological changes in cytokine-stimulated chondrocytes. Further work is necessary to identify the exact mechanism by which this calcium increase occurs.

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ENVIRONMENTAL POLLUTANTS AND OSTEOARTHRITIS: EFFECTS OF NON-DIOXIN-LIKE POLYCHLORINATED BIPHENYLS ON CULTURED CHONDROCYTES

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a. **Purpose**: Non-dioxin-like polychlorinated biphenyls (PCBs) are persistent organic pollutants that accumulate in fatty tissues causing immune suppression and endocrine disruption. Several studies suggest that PCBs may be involved in pathogenesis of osteoarthritis (OA). Alterations in the mechanisms of programmed cell death (apoptosis) are strongly related to the degradation of extracellular matrix (ECM) in the cartilage of OA subjects. Identification of apoptosis inducers is of paramount relevance to understand the pathogenesis and/or progression of OA. Thus, the aim of the present study was to assess the effect of several PCBs on chondrocytes viability and apoptosis induction.
b. **Methods**: The murine chondrogenic cell line ATDC5 and human juvenile costal chondrocyte cell line T/C-28a2 were treated with several doses of PCB 101, 153 and 180, alone and in combination. Cell viability was examined using a colorimetric assay based on the MTT labeling reagent. Apoptosis was evaluated by Annexin V flow cytometric assay and by the involvement of apoptotic related proteins, such as caspase-3 and Bcl-2/Bax ratio, using western blot analysis. Finally, to evaluate whether PCBs exert necrotic effect, apart from apoptosis pathways, we have also assessed lactate dehydrogenase (LDH) levels in culture supernatants.
c. **Results**: ATDC5 and T/C-28a2 cell lines treated with PCBs, alone and in combination, showed a significant reduction of cell viability rate in a concentration-dependent manner. Neither synergisms nor additive effects were observed on cell viability with the combined treatment. Data from annexin V assays suggested that PCBs clearly induced apoptotic pathways, as well as, a certain rate of necrosis. Actually, this effect was confirmed by evaluating LDH levels that were strongly increased in supernatants of PCBs-treated cells, suggesting that necrotic mechanisms are at play too. PCBs also induced caspase-3 activation by increasing its proteolytic cleavage in a concentration-dependent manner. Finally, the Bcl2/Bax ratio was also altered.
d. **Conclusions**: The viability of murine and human chondrocytes was reduced in presence of PCBs. The activity of PCBs on cell viability is likely to be mediated by alterations in the mechanisms of regulation of apoptosis and necrosis. Overall, this work highlights a novel role of environmental pollutants in the pathophysiology of chondrocytes.

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IL-1β MEDIATES MMP SECRETION AND IL-1B NEOSYNTHESIS VIA UPREGULATION OF $P22^{PHOX}$ AND NOX4 ACTIVITY IN HUMAN ARTICULAR CHONDROCYTES

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Purpose: Osteoarthritis (OA), the most common form of arthritis, results from the destabilization of the normal balance between the synthesis and the degradation pathways controlled by chondrocyte. That leads to a progressive degeneration of articular cartilage and subsequently to an alteration of the biochemical and biomechanical properties of the joint. Inflammation plays a major role in OA, particularly through the cytokine Interleukine-1β, promoting Reactive Oxygen Species (ROS) generation and Matrix Metalloproteinases (MMP) synthesis by the chondrocytes, which in turn orchestrate matrix proteolysis and catabolism. Nox4 belongs to the NADPH oxidase family whose function is to generate ROS. Nox4 associated with its stabilizing subunit $p22^{phox}$ is constitutively active. Given the critical role of oxidative stress in degenerative processes and in particular in OA, we assessed the role of NADPH oxidases in primary human articular chondrocytes (HAC) upon IL-1β stimulation.
Methods: Human articular chondrocytes (HAC) were isolated from femoral head of patients undergoing hip replacement. Production of ROS by Nox4 was measured by Amplex Red Assay. Effects of IL-1β on chondrocytes production of MMP-1, MMP-13, ADAMTS4, and IL-1β neosynthesis were assessed by quantitative RT-PCR and immunoblotting, in presence or not of Nox4 inhibitors.
Results: Our work demonstrates for the first time that Nox4 is expressed in HAC with $p22^{phox}$ and is a major source of ROS upon IL-1β treatment. Moreover, results show that ROS produced by Nox4 are critical mediators of IL-1β induced MMP-1, MMP-13 and ADAMTS4 synthesis and release. Interestingly, Nox4 activity inhibition by the Heme Oxygenase-1 (HO-1), the rate limiting step in heme catabolism, but also by pharmacological inhibitors (DPI or GKT) led to a significant decrease in MMP synthesis by HAC. It has been shown that IL-1β acts in an autocrine / paracrine manner leading to its own neosynthesis by HAC. Our results demonstrate the involvement of Nox4 in this autocrine loop and suggest that IL-1β stabilizes Nox4 expression/activity through an upregulation of $p22^{phox}$ in HAC and that upregulation of $p22^{phox}$ expression appears to be redox regulated in chondrocytes.
Conclusions: Finally, our data support a significant role for Nox4/$p22^{phox}$ in human articular chondrocytes mediating pro-catabolic pathways induced by IL-1β.

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EFFECTS OF PROSTAGLANDIN E2 ON SUPEROXIDE DISMUTASE GENE EXPRESSION IN CHONDROCYTE

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Inflammation is part of the complex biological response of tissues characterized by a cascade of biochemical events that propagates the inflammatory response where Prostaglandin and Superoxide Dismutase seems to be an important part of the key. Although Osteoarthritis is known as a degenerative disease, secondary inflammation may play an important role in the tissular changes that occurs in this disease. Chondrocyte is able to synthetize and react to most of intermediate of inflammatory agents. Prostaglandin may be overproduced in 20 fold by chondrocytes. Reactive oxygen species (ROS) are implicated in cellular inflammatory response, and Superoxide dismutase are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide; they are important antioxidant and antiimmflamatory defense in nearly all cells.
Purpose: The aims of this study was to further characterize the effects of Prostaglandin E2 on gene expression of Superoxide Dismutase (SOD) in bovine chondrocyte and ATDC-5 culture cells and determine their influence on inflammatory process in cartilage destruction.
Methods: After establishing the best conditions for cell culture, proliferation, toxicity and transfection, second step; the expression of SOD promoter constructs was analyzed in Bovine Chondrocytes and ATDC-5 cell. Briefly, cells were collected by centrifugation, resuspended in serum-free DMEM media, and then transfected with ExGen 500, a