Conclusions: This is the first study quantifying B2 receptors, the affinity and potency of BK and its antagonists in HCCK. Current data highlight the pharmacological profile of MEN16132 as a very potent bradykinin antagonist, in respect with icatibant, both in HFLS and HCCK.

433 MOLECULAR AND CELLULAR MECHANISMS REGULATING CROSS-TALK BETWEEN THE CYCLOOXYGENASE AND LIPOXYGENASE BIOSYNTHETIC AXES

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Purpose: Our principal objective is to define potential cellular and molecular interactions between infiltrating mast cells/mast-cell derived inflammatory mediators and resident human synovial fibroblasts ultimately contributing to disease pathophysiology.

Methods: 1. Cultured human synovial fibroblasts (HSF) were obtained from osteoarthritised-fected synovial membranes.
   2. Western and Northern blot analyses were used to measure protein and mRNA expression, respectively.
   3. Transient transfection assays were employed to express activated signaling molecules and/or dominant negative mutants and to analyze the reporter lucerase activity. A Mek1/2 inhibitor is also included in this reporter lucerase activity study.
   4. Prostaglandin E2 and leukotriene B4 levels were quantified by ELISA.
   5. Statistical analyses included Student's T-test and ANOVA.

Results: 1. Leukotriene B4 stabilized cyclooxygenase-2 mRNA and cyclooxygenase-2 protein levels in II-1]-treated human synovial fibroblasts, leading to the increase of prostaglandin E2 production.
   2. Leukotriene B4 stabilized cyclooxygenase-2 mRNA on post-transcriptional level through the cyclooxygenase-2 mRNA 3'-UTR region in II-1]-treated human synovial fibroblasts. There is no transcriptional regulation in this stabilization effect of leukotriene B4.
   3. Leukotriene B4 exerts this stabilization through Raf/MEK1/ERK1/2 signaling pathway in II-1]-treated human synovial fibroblasts.
   4. The AU-containing proximal region of cyclooxygenase-2 mRNA 3'-UTR is more important for this stabilization effect of leukotriene B4 in II-1]-treated human synovial fibroblasts than more distal AU rich sequences.

Conclusions: The pathogenic mechanisms responsible for arthritis remain poorly understood both systemically and in the microenvironment of the diarthrodial joint. Mast cells are detected in the synovial membrane of osteoarthritic patients. We demonstrate the potential cellular and molecular interactions between mast cells and mast-cell derived inflammatory mediators and resident human synovial fibroblasts. We hypothesize that mast-cell derived leukotrienes contribute to synovial inflammation through stabilization of synovocyte cyclooxygenase-2 expression.

434 ELECTROMAGNETIC FIELDS AND ADENOSINE RECEPTORS IN CHONDROCYTES AND SYNOVIAL FIBROBLASTS

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Purpose: Electromagnetic fields (EMFs) stimulate anabolic activities in cartilage explants and prevent the catabolic effect of the inflammatory cytokine II-1. In vivo EMFs retard the development of OA lesions in guinea pigs; in humans they have been successfully used for the treatment of OA. Adenosine is known to reduce inflammation in several models by interacting with four receptors (A1, A2A, A2B and A3). The role of adenosine for managing joint inflammation has been recently documented and drugs with adenosine A2A receptor agonist activity have shown chondroprotective effects. The aim of this study was to investigate the potential anti-inflammatory activities of EMFs by characterizing their effects on adenosine receptors expression in bovine chondrocytes and SFs. The functional interaction among adenosine A2A analogs and EMFs in SFs was analyzed by evaluating the production of cAMP, an intracellular mediator, the release of prostaglandin E2 (PGE2) and cyclooxygenase-2 (COX-2) expression.

Methods: Chondrocytes and SFs isolated from bovine articular joints were cultured in vitro. The effects of EMFs (1.5 mT, 75 Hz) on adenosine receptors were investigated by saturation and competition binding experiments. Adenyl cyclase assays were performed to evaluate cAMP levels induced by the A2A agonist, CGS 21680 (2-[p-(2-carboxyethyl)-phenetyl-amino]-5'-N-ethyl-carboxamido adenosine) and the A2B agonist, NECA (5'-N-ethyl-carboxamido adenosine) in the absence and in the presence of EMFs. CGS 21680 and NECA were added to SFs untreated or treated with TNF-alpha (10 ng/ml) in the absence or in the presence of EMFs. PGE2 release was measured by immunoassay and COX-2 expression was evaluated by RT-PCR.

Results: EMFs evoke the upregulation of the A2A and A3 receptors in both SFs and chondrocytes. The increase in A3 receptor density (1.9 and 2.4 folds, p < 0.01 respectively in chondrocytes and SFs) was associated to an increase in cAMP levels indicating the functionality of the receptors in EMF-exposed cells. TNF-alpha enhanced PGE2 release from SFs. CGS 21680 and NECA significantly inhibited PGE2 production respectively of 49% and 55%, EMFs induced PGE2 release of 63% (vs. TNF-alpha, p < 0.01) and strongly enhanced the inhibition induced by the agonists. Modification of COX-2 expression mirrored changes in PGE2 levels.

Conclusions: This study supports anti-inflammatory activity of EMFs mediated by an up-regulation of A2A receptors and shows a molecular action mechanism by which EMFs act. In vitro EMF anti-inflammatory activity is consistent with the decrease in inflammatory cytokines expression in the articular cartilage of OA animals. Noteworthy, the biophysical modulation of adenosine pathways has been used in humans as a therapeutic intervention to control the inflammatory microenvironment in arthrosopic procedures.

435 ROLE OF HEME OXYGENASE-1 IN THE CONTROL OF HIGH MOBILITY GROUP BOX CHROMOSOMAL PROTEIN 1 IN OSTEOARTHRITIC SYNOVIOCYTES

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Purpose: The production of inflammatory mediators may contribute to osteoarthritis (OA) pathology. High mobility group box chromosomal protein 1 (HMGB1) acts as a pro-inflammatory cytokine in a wide range of cells and binds to the receptor for advanced glycation products (RAGE), a pathway leading to catabolic responses in articular tissues. Of particular interest in the regulation of inflammatory and catabolic processes is the
heme oxygenase-1 (HO-1) pathway, which is able to counteract cellular stress in vitro and in vivo. The present study was aimed at identifying a possible regulatory effect of HO-1 on HMGB1 in OA synoviocytes.

Methods: Synovial tissue samples were obtained from 15 OA patients undergoing total knee joint replacement. Synoviocytes (fibroblasts and macrophage-like) were obtained by digestion with collagenase IA, cultured in tissue culture and treated with interleukin-1β (IL-1β; 100 U/ml) for 24 h. HO-1 was induced by treatment with cobalt protoporphyrin IX (CoPPIX; 10 μM). Matrix metalloproteinase (MMP) activity was determined by fluorometric procedures and HMGB1 release by ELISA. Protein expression was studied by Western blot.

Results: Basal expression of HMGB1 protein was reduced by HO-1 induction in OA synoviocytes. In addition, HMGB1 release into the medium was significantly decreased. Stimulation of synoviocytes with IL-1β resulted in an enhancement of HMGB1 cellular content and release. Our results indicate that both processes are down-regulated by HO-1 overexpression. In addition, HO-1 reduced RAGE expression in these cells. The effects of HO-1 induction were prevented when synoviocytes were transfected with a siRNA specific for human HO-1. In cells without HO-1 induction, HO-1 gene silencing resulted in the up-regulation of HMGB1 and RAGE. Regulation of HMGB1 by HO-1 was assessed by the inhibition of MMP activity in synoviocytes stimulated with IL-1β.

Conclusions: Our data provide evidence that HO-1 can regulate HMGB1 in OA synoviocytes. Overall, HO-1 signaling appears to be an appropriate target for the development of novel therapies affecting arthritic disorders.

Effect of Epigallocatechin Gallate on the Inflammatory Response of IL-1-Exposed Synovial Fibroblasts

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Purpose: Inflammation is increasingly recognized as contributing to the symptoms and progression of osteoarthritis (OA). Synovitis is a factor that likely contributes to dysregulation of chondrocyte function, favoring an imbalance between the catabolic and anabolic activities of the chondrocyte in remodeling the cartilage. In recent years, significant interest has emerged in the beneficial health effects attributed to the green tea polyphenols. Polyphenols in green tea are potent antioxidants, with the majority of the beneficial effects elicited by epigallocatechin-3-gallate (EGCG), one of the main constituents of green tea. The aim of our study was to evaluate if EGCG may influence some inflammatory aspects of OA. To this aim we studied the effect of EGCG on chemotactic factors released by human fibroblasts stimulated with calcium crystals, regular features of the most severe forms of OA.

Methods: Human synovial fibroblasts were stimulated with pyrophosphate dihydrate (CPPD) and basic calcium phosphate (BCP) crystals (0.01–0.1 mg/ml) in the presence or absence of EGCG (0.1–5 μM). IL-1β (10 ng/ml) was used as a positive control. CPPD and BCP crystals were synthesized by the methods of Cheng and McCarthy respectively. The levels of MCP-1 were measured in cell supernatants by enzyme-linked immunosorbent assay methods. The chemotactic effect of culture supernatants was evaluated on chemotaxis chamber by the migration of fresh-isolated mononuclear blood cells. The lack of cell cytotoxicity of both EGCG and calcium crystals was ensured using the colorimetric MTT assay.

Results: EGCG inhibited MCP-1 release by stimulated fibroblasts in a dose-dependent manner. Supernatants of crystals-stimulated cells lose their ability to induce mononuclear cell migration when EGCG was added in the medium. EGCG inhibited both MCP-1 release and supernatants chemotactic activity of IL-1β stimulated culture in a dose-dependent manner.

Conclusions: The present study shows that EGCG, at dose comparable with plasma concentration achieved by the consumption of two cups of tea, modifies the inflammatory response of calcium crystal-exposed synovial fibroblasts. EGCG interferes with inflammatory signal transduction pathway and may also inhibit the cellular generation, the release and the accumulation of reactive oxygen species. Our results suggest that EGCG might represent a good candidate for the prevention and treatment of OA.

Effect of Epigallocatechin Gallate on the Secretion of Chemotactic Factors by Calcium Crystal-Induced Inflammasome in OA Synoviocytes

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Purpose: Although osteoarthritis (OA) is defined as a cartilage disease, synovitis involving mononuclear cell infiltration and overexpression of proinflammatory mediators is common in early and late OA. Calcium crystals deposition is a factor that likely contributes to synovial membrane inflammation. Polyphenols in green tea are potent antioxidants, with the majority of the beneficial effects elicited by epigallocatechin-3-gallate (EGCG), one of the main constituents of green tea. The aim of our study was to evaluate if EGCG may influence some inflammatory aspects of OA. To this aim we studied the effect of EGCG on chemotactic factors released by human fibroblasts stimulated with calcium crystals, regular features of the most severe forms of OA.

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