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**BRADYKININ VIA THE B₂ RECEPTOR SYNERGISTICALLY POTENTIATES THE INTERLEUKIN 1β ACTIVATED RELEASE OF PROSTAGLANDIN E₂ AND CYCLOOXYGENASE 2 EXPRESSION IN HUMAN-FIBROBLAST LIKE SYNOVIOCYTES**

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**Purpose:** Interleukin 1β (IL-1β) is considered one of the key players in osteoarthritis (OA) pathogenesis. It increases the transcription of several mediators and enzymes, amongst which the inducible cyclooxygenase isoform COX2. Bradykinin (BK, H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH) is a proinflammatory and algogenic peptide which is enzymatically generated from kininogen precursors circulating in plasma and interstitial fluids. BK, also detected in the synovial fluid, activates B₂ receptors present on the membrane of fibroblast-like synoviocytes to release prostaglandins, cytokines and chemokines. Aim of this study was to investigate the interaction of BK with IL-1β, at concentrations resembling those measured in the OA synovial fluid, and to evaluate the effect of BK B₂ receptor blockade, by using the highly selective and potent B₂ receptor antagonist MEN16132 (m.w. 873.16).

**Methods:** Human synoviocytes (ECACC, 408-05a) were cultured in synoviocyte growth medium (ECACC, 06091516) supplemented with Gln 2mM, penicillin (50 μg/ml), streptomycin (50 μg/ml), amphotericin B (0.75 μg/ml). Experiments were performed with cells at confluence plated onto 12-well plates. Cells were incubated at the indicated compound concentration in F12 medium supplemented with foetal bovine serum (1%), Gln (2 mM), and captoril (1 μM). At the end of the experiments, supernatants were stored at -80°C and used for PGE₂ content detection (Cayman, 514010), whereas cells were collected for RNA extraction and following qPCR COX2 gene expression. Data are expressed as -fold increase versus untreated cells (basal) and represent the mean ± s.e.m. of 3-4 experiments, each in duplicate.

**Results:** A prolonged incubation (24h) of synoviocytes with BK (1 μM) induced a significant production of PGE₂ (3.2±0.8 -fold of basal) and COX2 gene expression (1.9±0.3 -fold of basal). However, PGE₂ production and COX2 gene expression were reduced by the pretreatment (30 min) with the B₂ receptor antagonist MEN16132 (1 μM) (PGE₂: 1.6±0.3; COX2: 0.7±0.1 -fold of basal), which per se did not affect the basal release of PGE₂ (1.1±0.2 -fold of basal) or the COX2 gene expression (0.9±0.2 -fold of basal). In contrast, the effect of IL-1β (15 pg/ml) was significantly more pronounced, since it augmented the PGE₂ release by 44.7±13.3 -fold and the COX2 gene expression by 11.2±2.1 -fold in respect to basal values obtained in control cells. The combined treatment of cells with BK and IL-1β induced an even increase, both in terms of released PGE₂ (148.0±35.4 -fold of basal) and COX2 gene expression (28.2±4.9 -fold of basal), which indicated a synergistic rather than an additive effect. These potentiating effects of BK on both the PGE₂ production and increased COX2 gene expression produced by IL-1β were B₂ receptor mediated since the pretreatment with the B₂ receptor antagonist could prevent it (PGE₂: 54.6±7.3; COX2: 14.0±2.5 -fold of basal).

**Conclusions:** These results indicate that BK has potentiating effect on the COX2 gene expression and consequent prostaglandin production induced by IL-1β, and suggest that B₂ receptor blockade by MEN16132 may represent a potential symptomatic therapy for OA.

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**REGULATION OF INFLAMMATORY MEDIATORS BY HEME OXYGENASE-1 IN HUMAN OSTEOSTRORHIC OSTEOBLASTS**

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**Purpose:** Alterations in osteoblast metabolism may play a role in osteoarthritis (OA). We have shown previously that heme oxygenase-1 (HO-1) exerts protective effects in cartilage by decreasing the production of inflammatory and degenerative mediators. In this study, we have investigated the role of HO-1 in the regulation of the inflammatory response in OA osteoblasts.

**Methods:** Osteoblasts were obtained from 7 OA patients undergoing total knee joint replacement. Bone tissue from the trabecular area of the tibial plateau was minced into small portions and digested with collagenase IA for 2 h under agitation. Samples were then seeded in 100 mm petri dishes in osteogenic medium. Bone pieces were removed and osteoblasts were allowed to grow until third passage. Cells were treated with the HO-1 inducer cobalt protoporphyrin IX (CoPP, 10 μM) in the presence or absence of interleukin-1β (IL-1β, 10 ng/ml) for 3 h (mRNA determination) or 24 h (other determinations). Matrix mineralization was determined by Alizarin Red incorporation. Gene expression was analyzed by quantitative PCR and protein expression by Western Blot and ELISA. Matrix metalloproteinase (MMP) activity was determined by fluorometric procedures and PGE₂ by RIA.

**Results:** IL-1β inhibited protein expression of HO-1 in osteoblasts with respect to basal conditions. This pro-inflammatory cytokine significantly increased MMP activity and PGE₂ production. These effects were significantly reverted by CoPP treatment. PCR analysis showed the down-regulation of HO-1 mRNA induced by IL-1β, and the up-regulation of COX-2, mPGES-1, MMP-1, MMP-2, MMP-3, IL-6, HMGB-1 and RAGE. CoPP was also able to significantly revert these effects of IL-1β. Inhibition of PGE₂ production by CoPP would be dependent on the decreased expression of COX-2 and mPGES-1, a key mediator of bone destruction associated with inflammation.

**Conclusions:** In this work we have shown that HO-1 decreases the production of relevant inflammatory and catabolic mediators that participate in OA pathophysiology. Our data suggest that HO-1 could be a pharmacological target to control the activity of osteoblasts.

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**EFFECT OF IL-1β ON THE PROTEOME OF CHONDROCYTES DERIVED FROM HUMAN OSTEOSTRORHIC CARTILAGE-A PHARMACOPROTEOMICS APPROACH FOR DRUG SCREENING**

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**Purpose:** Inflammation plays a pivotal role in cartilage tissue destruction in osteoarthritis. IL-1β, a key mediator, affects the balance of biosynthesis and degradation of extracellular matrix (ECM) constituents by chondrocytes. Indeed, exuberant synthesis of matrix metalloproteinases and reduced expression of collagen II and aggrecan are well-known cytokine-mediated hallmarks of osteoarthritis. In addition, IL-1β induces the production of the pain mediator prostaglandin E2 via cyclooxygenase which decisively contributes to joint dysfunction. Today’s treatment options are restricted to symptome-modifying drugs. However, analogics or anti-inflammatory drugs partially show just limited efficacy with respect to pain relief or cause undesirable side effects. Hence, the identification of disease-modifying and efficient symptome-modifying drugs is a main challenge in osteoarthritis research.

**Methods:** Pharmacoproteomics is a promising approach for drug screening. In order to establish a reference system for monitoring substance effects, we performed proteome profiling of human osteoarthritis chondrocytes. Alterations of the secretion performance and the metabolism of chondrocytes due to IL-1β treatment were assessed by 2D-PAGE and shotgun proteomics combined with the mass spectrometrical identification of proteins.

**Results:** A special focus was laid on the determination of interindividual differences existing per se as well as those arising due to stimulation by IL-1β. The proteomes were functionally characterized and biomarkers/functions reflecting the cytokine effect were gathered. We identified well-known markers for cartilage degradation such as MMP-1 and IL-6, IL-8 among many others in the context of a global cytokine-induced alteration of gene expression. The resulting database was used to assess the effect of different derivatives of hyaluronic acid in order to evaluate the applicability of this approach.

**Conclusions:** Our approach enabled insights into molecular alterations due to cytokine and substance effects at a comprehensive systemic level. Furthermore, it proved to be well-suited to identify ‘real’ substance effects against the background of the patient- and disease-derived heterogeneity of samples.