455

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

S. Meini1, P. Cucchi1, L. Tinti2, S. Niccolini2, L. Muscar1, F. Bellucci1, C. Catalani1, S. Giuliani1, M. Galeazzi2, A. Fioravanti2, C. Maggi1
Menarini Ricerche S.p.A., Florence, Italy; 2Univ. of Siena, Siena, Italy

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 cycloxygenase 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.

Methods: Human synoviocytes (ECACC, 408-05a) were cultured in synoviocyte growth medium (ECACC, 06091516) supplemented with Glu 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 captopril (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 the PGE2 production and increased COX2 gene expression produced by IL-1β were B2 receptor mediated as such 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 prostanooid production induced by IL-1β, and suggest that B2 receptor blockade by MEN16132 may represent a potential symptomatic therapy for OA.

457

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

H. Zwickl1, E. Niculescu-Morza2, F. Hallbworth1, V. Haudek2, C. Gerner2, S. Nehrer1
1Ctr. for Regenerative Med., Krems, Austria; 2Inst. of Cancer Res., Medical University Vienna, Austria

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, analgesics or anti-inflammatory drugs partially show only 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 osteoarthritic 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-induces 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.