Effects of Pulsed Electromagnetic Field Exposure and Adenosine Analogues on Prostaglandin Release in Bovine Synovial Fibroblasts

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Introduction: Chronic inflammation plays a key role in Osteoarthritis (OA) and Rheumatoid arthritis (RA). Synovial fibroblasts (SFs) are thought to be central to the pathogenesis of these joint diseases by producing matrix degrading enzymes, cytokines and lipid inflammatory mediators such as prostaglandins (PGE). Biophysical stimulation with pulsed electromagnetic fields (PEMFs) appears as a potential therapeutic approach to control inflammation and manage joint diseases as suggested by the results of several studies. In cartilage explants cultured in vitro, PEMFs increase proteoglycan synthesis preventing the catabolic effect of the pro-inflammatory cytokine IL-1 and acting in synergy with insulin-like growth factor I (I). In vivo, PEMFs preserve the morphology of articular cartilage and retard the development of osteoarthritic lesions in guinea pig and in clinics they have been successfully used for the treatment of osteoarthritis (OA) (2). However, to date little is known about PEMF action mechanisms. Recently, it has been demonstrated that PEMFs evoke an upregulation of the A2A and A3 adenosine receptors in human neutrophils and bovine synovial fibroblasts and chondrocytes (3, 4). Adenosine, interacting with four types of cell surface G-protein-coupled adenosine receptor (AR) proteins, termed A1, A2A, A2B, and A3 acts as a potent endogenous inhibitor of inflammatory processes in several tissues. In cartilage adenosine depletion increases glycosaminoglycan release and the production of matrix metalloproteinases, PGE2 and nitric oxide (NO) (5). In vivo adenosine A2A receptor agonists inhibit cartilage damage in septic arthritis. In this study we analyzed the effects on the synthesis of prostaglandin E2 (PGE2), the main prostaglandin produced by SFs in response to inflammatory stimuli.

Materials and Methods: SFs were obtained by bovine synovial fluid and cultured in monolayer in complete medium (Dulbecco’s modified Eagle’s/Ham’s F12 (1:1) medium (DMEM/F12) supplemented with 10% FBS. SFs at the 3rd-4th passage were plated at 10000/cm² in multiwells (Nunc, Denmark, 6.6 X 6.6 cm, 1.6 cm the diameter of each well). Known pro-inflammatory stimuli including the TNF-alpha (10 ng/ml) and the bacterial lipopolysaccharides (LPS) (1 μg/ml) were used to activate the inflammatory process in SFs. Adenosine synthetic analogues were added to both control and TNF-alpha- or LPS-treated cultures. Adenosine analogues used include: the A2 and A1 receptor agonist 5’-N-ethylcarboxamido-2-chloroadenosine (NECA), the A1 agonist N6-cyclohexyladenosine (CHA), the A2A agonist 2’-[p-(2-carboxylethyl)-(phenethyl-aminoo)-5’-N-ethylcarboxamido adenosine (CGS 21680) and the A3 agonist N6-(3-iodobenzyl)2-chloroadenosine-5’-N-methyluronamide (CI-IB-MECA). All treatments were performed both in the absence and in the presence of 2 IU/ml adenosine deaminase (ADA) used to deplete endogenous adenosine. Parallel cultures were exposed to PEMF (75 Hz, 1.5 mT) (Igea, Carpi, Italy) during the whole period in culture (24h). The release of PGE2 was measured using a commercially available competitive enzyme immunoassay and data were expressed as pg PGE2/μg protein.

Results: Treatment of cells with CHA, CGS 21680, CI-IB-MECA and NECA did not modify basal PGE2 production. Similarly, PEMF exposure did not modify PGE2 production in unstimulated SFs, both in the absence and in the presence of the adenosine receptor agonists. TNF-alpha and LPS strongly stimulated PGE2 production yielding a maximal 7.9 and 9.8 fold increase, respectively. All adenosine agonists, except for CI-IB-MECA, significantly inhibited PGE2 production to a similar degree. PEMF exposure significantly inhibited PGE2 production in the absence of adenosine agonists (61.4 % vs. TNF-alpha and 51.5 % vs. LPS) and increased the inhibitory effects of CHA, CGS and NECA. In 2 IU/ml ADA, TNF-alpha and LPS stimulated PGE2 synthesis similarly to what observed in the absence of ADA. The inhibition on PGE2 production induced by CHA, CGS and NECA was stronger than in the absence of ADA and PGE2 levels did not differ from control cells. In this experimental condition the inhibitory effect of PEMF exposure alone on PGE2 production was significantly reduced in TNF-alpha and LPS treated SFs.

Discussion: Results of this study show that both PEMFs and the activation of adenosine receptors can strongly inhibit the release of PGE2 in SFs. Further they show the specific involvement of A1 and A2A adenosine receptor activation in the inhibition of PGE2 production. These results are in line with the known anti-inflammatory activities of adenosine. The increased activity of adenosine agonists induced by PEMFs and the reduced activity of PEMFs in the absence of adenosine strongly support the hypothesis that PEMFs can act by the activation from endogenous adenosine on A2A upregulated adenosine receptors (4). In conclusion in this study we show that PEMFs can modulate inflammatory activities in SFs and indicate for the first time a molecular action mechanism by which PEMFs can act. The involvement of adenosine receptors and PEMF exposure in the inflammatory responses of synovial fibroblasts open perspectives to develop novel anti-inflammatory approaches in degenerative diseases for the joint preservation.