Relevance to Musculoskeletal Conditions

This study examined the effect of prostaglandin E2 (PGE2) on basic fibroblast growth factor (bFGF) mRNA expression of rheumatoid synovial fibroblasts, and the role of PGE receptors (EP receptors) linked to bFGF mRNA expression in synovial fibroblasts, which might develop the therapeutic effect of EP receptor antagonist on rheumatoid arthritis.

Introduction

In rheumatoid arthritis (RA), it is characterized that pronounced synovial hyperplasia composed of the extensive proliferation of synovial fibroblasts (SFBs) and inflammatory cell infiltration with neovascularization. Pannus tissue that is the hyperplastic synovium invades both surface of articular cartilage and subchondral bone adjacent to the synovial-cartilage junction. It secretes a variety of growth factors including basic fibroblast growth factor (bFGF) and inflammatory cytokines, which stimulate the growth of pannus tissue itself. Inflammatory cytokines such as interleukin-1 stimulate the prostaglandin E2 (PGE2) synthesis of synovial cells and thus PGE2 is detectable at a high level in the fluid of knee joints in OA and RA.

It is recently reported that PGE2 increases bFGF mRNA expression in rat Müller cells and bovine adrenal medullary cells. Basic FGF seems to be involved in synovial hyperplasia, neovascularization and joint destruction in RA. FGFs also play a critical role in the bone remodeling including bone formation and resorption. It is reported that bFGF at low concentrations acts directly on mature osteoclasts to resorb bone moderately, whereas at high concentrations it acts on osteoblastic cells to induce cyclooxygenase-2 and stimulates bone resorption potently. These findings suppose that PGE2 might be related to the exacerbation of joint inflammation and involved in the disease process of degenerative arthritis through bFGF synthesis.

On the other hand, PGE2 is reported to be an antiproliferative molecule. For instance, PGE2 suppressed RA synovial cell proliferation directly through intracellular cAMP accumulation. Thus, the role of PGE2 in inflammatory process is still elusive. We have, therefore, studied the effect of PGE2 on bFGF mRNA expression of rheumatoid SFBs.

Material and Methods

Cell culture

Synovial tissue samples were surgically obtained, after consent, from patients with RA at the time of total knee arthroplasty. The dissociated cells by collagenase were suspended in Dulbecco's modified Eagle's medium supplemented with 10% heat inactivated fetal bovine serum and cultured in a tissue culture flask. When the primary culture reached confluence, culture medium was supplemented with 10% heat inactivated fetal bovine serum and cultured in a tissue culture flask. When the primary culture reached confluence, culture medium was supplemented with 10% heat inactivated fetal bovine serum and cultured in a tissue culture flask.

Preparation of total RNA and RT-PCR

In order to study whether PGE2 induces bFGF synthesis of SFBs, the expression of bFGF mRNA in SFB was detected using RT-PCR. SFBs were plated at 1.0×10⁵ cells per well in 6-well plates and stimulated PGE2 for 6 h. Total RNA was directly isolated from the cell monolayer using a RNeasy Mini kit according to the manufacturer's instructions. Complementary DNA reverse-transcribed using oligo-dT primer from total RNA was amplified by specific primers for either human bFGF or human GAPDH. A fraction of each PCR products was then electrophoresed in a 1.5% agarose gel followed by ethidium bromide staining for visualization.

Results and discussion

We have already reported that over 10⁻⁶ M of PGE2 could suppress the proliferation of SFBs. This suppression of cell growth is supposed to be mediated by intracellular cAMP elevation due to EP2 or EP4 receptor activation, because both EP2/EP4 receptor agonists (butaprost/17-PGE1-OH) and direct adenylate cyclase activator, forskolin has shown the antiproliferative effect of SFBs. On the other hand, neither EP1 receptor agonist, 17-phenyl-trior PGE2 nor EP3 receptor agonist, sulprostone affect cell proliferation up to 10⁻⁶ M. Thus, PGE2 is seemed to have an antiproliferative property on SFB through EP2/EP4 receptors.

In terms of bFGF mRNA expression, it is recently reported that EP2 receptor activation was required in bFGF mRNA expression of rat Müller cells and bovine adrenal medullary cells. Therefore, the effect of PGE2 on bFGF mRNA expression of SFB was investigated. As shown in Fig.1, bFGF mRNA expression was markedly enhanced when SFBs were treated with 10⁻⁶ M of PGE2. Physiological functions of PGE2 are thought to be mediated through interactions with four distinct prostaglandin receptors, EP1, EP2, EP3, and EP4, which are associated with different signal transduction pathways. Surprisingly, EP1 receptor agonist, 17-phenyl-trior PGE2 has enhanced bFGF mRNA expression, whereas EP2, EP3 and EP4 receptor agonist failed to augment mRNA expression shown in Fig. 2. As the activation of EP1 receptor has been associated with a rise in intracellular calcium, the effect of calcium ionophore, A-23187 on bFGF mRNA expression of SFBs was also examined. 10⁻⁶ M of A-23187 augmented bFGF mRNA expression of SFBs, whereas forskolin failed to do that. These observations imply the effect of PGE2 on bFGF mRNA expression is mediated through EP1 receptor not through EP2 and EP4.

Further investigation should be required for detecting the exact receptor engaged in bFGF synthesis of SFB in RA. It is supposed that EP1 antagonist may utilize for decreasing bFGF synthesis to suppress pannus formation and prevent bone resorption led to joint destruction.

Conclusion

PGE2 induce bFGF mRNA expression through EP1 receptor activation and development of antagonistic analogue for EP1 receptor might contribute the RA therapy by suppressing bFGF synthesis.