Relevance to Musculoskeletal Conditions

This study examined the type of prostaglandin (PG) receptors expressed on synovial fibroblasts and the role of these receptors linked to antiproliferative effect of PGE2 on rheumatoid synovial fibroblasts, which might develop the therapeutic effect of PGs on rheumatoid arthritis.

Introduction

ProstaglandinE2 (PGE2) is detectable at a high level in the fluid of knee joints in osteoarthritis and rheumatoid arthritis (RA). PGs are synthesized from eicosatetraenoic acids in the presence of cyclooxygenase (COX). The expression of the inducible COX isoform, COX-2, but not constitutive form, COX-1, was found to be elevated in a disease-related pattern in the synovial tissue from patients with RA in comparison with OA. It is also reported that cartilage specimens from OA-affected patients spontaneously released PGE2 at levels higher than in cytokine-treated normal cartilage due to upregulation of COX-2. These results suggested that PGE2 might exacerbate joint inflammation and be involved in the disease process of degenerative arthritis.

On the other hand, PGE2 is reported to be an antiproliferative molecule and one of the inducers of apoptotic change in various types of cells. For instance, PGE2 induces cAMP accumulation and inhibits the growth of the most differentiated breast cancer cells due to loss and probably dysfunction of PGE2 receptors. PGE2 also suppressed RA synovial cell proliferation through intracellular cAMP dependent mechanism. Thus, role of PGE2 in inflammatory arthritis is still elusive and should be discussed. Therefore, we have investigated the type of PGE receptor (EP receptor) on RA synovial fibroblasts linked to antiproliferative effect of PGE2 in order to discover the new therapeutical aspect of PGs and PG analogues.

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 enzymatically were suspended in Dulbecco’s modified Eagle’s medium supplemented with 10% heat inactivated fetal bovine serum and cultured in tissue culture flask. When the primary culture reached confluency, culture flasks were vigorously rinsed to remove non-adherent cells, and resultant adherent synovial fibroblasts (SFBs) were then used in the second or third passage for the experiments described below.

Assay for tritiated thymidine ([3H]-TdR) incorporation into SFBs.

In order to examine the effect of PGE2 or selective agonists of EP receptors on DNA synthesis of SFB, [3H]-TdR incorporation into the SFB was quantified.

Preparation of total RNA and RT-PCR.

For detecting the expression of EP receptors on RA-SFBs, EP receptor mRNA in RA-SFB was detected using RT-PCR with specific primers. RA-SFB were plated at 1.0×10⁵ cells per well in 6-well plates and stimulated by varying concentration of interleukin-1β (IL-1β) for 4 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 and a fraction of each PCR products was electrophoresed in a 1.5% agarose gel followed by cybergreen staining and fluorescent intensity was compared with expression of GAPDH mRNA.

Results and discussion

When RA-SFBs were cultured with varying concentration of PGE2 for 72 h incubation, proliferation of SFBs was suppressed in a dose dependent fashion. Over 10⁻¹⁰ M of PGE2 has shown significant decrease of [3H]-TdR incorporation in RA-SFB monolayer culture (Fig. 1). The actions of PGE2 are mediated by four distinct classes of EP receptors (EP1 through EP4). PGE2 is reported to suppress RA synovial cell proliferation through intracellular cAMP dependent mechanism. Increased cAMP generation by PGE2 may be due to EP2 or EP4 receptor activation. Therefore, EP2 or EP4 receptor is supposed to be expressed on the surface of RA-SFBs. However, it is still unclear which receptors are expressed on the surface of RA-SFBs. Therefore, effect of selective agonists of EP receptors on RA-SFB proliferation was examined. Effect of selective agonists of EP receptors on RA-SFB proliferation was examined.

Conclusion

PGE2 or its analogue agonistic for EP2 or EP4 receptor might contribute the RA therapy by suppressing pannus formation.