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Effect of Cyclooxygenase Inhibitor on Rheumatoid Synovial Fibroblasts Proliferation in Vitro

Ryuichi Saura¹, Soichiro Hirata¹, Hitoshi Ishikawa¹, and Kosaku Mizuno²

In order to examine the antirheumatic effect of nonsteroidal antiinflammatory drugs (NSAIDs), rheumatoid arthritis (RA) synovial fibroblasts were cultured with cyclooxygenase (COX) inhibitor resveratrol (specific for COX-1) or NS-398 (specific for COX-2) in vitro. Resveratrol augment cell proliferation, whereas NS-398 suppressed it. Prostaglandin E₂ reversed this resveratrol induced cell proliferation to basal level. These results suggested that selective COX-2 inhibitor may inhibit the pannus formation and suppress the progression of RA joint destruction.

Key Words
cyclooxygenase (COX), prostaglandin, nonsteroidal anti-inflammatory drugs (NSAIDs), rheumatoid arthritis, joint destruction, chemoprevention.

Introduction

In rheumatoid arthritis (RA), synovial neovascularization and proliferation of synovial fibroblasts have a critical role in the propagation of rheumatoid synovitis. Disease modifying anti-rheumatic drugs (DMARDs) such as gold sodium thiomalate, D-penicillamine and methotrexate has been reported to have beneficial effects on RA through the anti-proliferative effect of either endothelial cells or synovial fibroblasts¹-³. Also, nonsteroidal anti-inflammatory drugs (NSAIDs) have beneficial effects on inflammatory arthritis through the inhibition of prostaglandins (PGs) synthesis. Recently, it is reported that cyclooxygenase (COX)-2, which are key enzymes in PG synthesis, may be related to carcinogenesis of colon⁴ and the administration of NSAIDs may reduce the risk of fatal colon cancer⁵,⁶. Cyclooxygenase-2 is also suggested to play an important role in RA synovial cell growth required for pannus formation⁷. However, it is unclear whether administration of NSAIDs might prevent joint destruction through the suppression of COX-2 activities in RA. In the present study, we have, therefore, examined the effect of COX inhibitors on RA synovial cell proliferation in vitro.

Materials and methods

1. Preparation of synovial fibroblasts (SFBs) and SFB monolayers
   Synovial tissue samples were surgically
obtained, after consent, from patients with RA at the time of total knee arthroplasty. Synovial fibroblasts were isolated as described previously with some modification\(^8\). Briefly minced synovial tissue were enzymatically dissociated using 0.2% of collagenase (Sigma Chemical Co., St. Louis, MO, USA) and 0.25% of trypsin-EDTA (DIFCO LABORATORIES, Detroit, MI, USA) at 37° C for 2 hours, with gentle stirring. The dissociated cells were suspended in Dulbecco’s modified Eagle’s medium (DMEM; Gibco BRL., Grand Island, NY, USA) supplemented with 10% heat inactivated fetal bovine serum (FBS; Biowhittaker., Walkersville, ML USA), 100 units/ml penicillin and 100 \(\mu\)g/ml streptomycin and cultured in tissue culture flask (Corning Incorporated, Coming, NY, USA) at cell density of \(10^5\) cell/cm\(^2\).

When the primary culture reached confluency, culture flasks were vigorously rinsed to remove non-adherent cells, and resultant adherent synovial fibroblasts were then used in the second or third passage for the experiments described below.

2. Assay for tritiated thymidine ([\(^3\)H]-TdR) incorporation into SFB

In order to examine the effect of COX inhibitors on DNA synthesis of SFB, [\(^3\)H]-TdR incorporation into the SFB was quantified. Ten thousand of SFBs were allowed to attach in every well of 96-well flat bottomed culture plates (Corning Incorporated, Corning, NY, USA) in DMEM with 10% FBS and cultured for 72 hours. Fifteen hours before terminating proliferation assay of incubation time, 37 KBq of [\(^3\)H]-TdR were added to each well. At the end of proliferation assay, SFB were washed three times with distilled water. Two hundred microliter of scintillation liquid were added and cell associated [\(^3\)H]-TdR was determined using scintillation counting (PACKARD INSTRUMENT COMPANY, Mariden, CT, USA).

3. Statistical analysis

Statistical analysis was carried out on all data points with regard to control by a Mann–Whitney’s U test. Each data point represented the mean of four separate samples with the corresponding standard error of the mean (SEM). P values under 0.05 were considered significant, statistically seen.

Results

1. Effects of COX–1/2 selective inhibitors on [\(^3\)H] TdR incorporation into RA synovial fibroblasts

When RA synovial fibroblasts were cultured for 72 hours in the presence of COX–1 selective inhibitor, resveratrol\(^9\), [\(^3\)H]–TdR incorporation into cells were enhanced in a dose dependent fashion. Significant increase of [\(^3\)H]–TdR incorporation was observed at the concentration of over 0.5 \(\mu\)g/ml resveratrol (Fig. 1 a). In contrast, selective COX–2 inhibitor, NS–398\(^{10}\) inhibited cell proliferation indicated by [\(^3\)H] TdR incorporation into cells dose-dependently. Significant inhibition of cell proliferation was observed at the concentration of over 25 \(\mu\)g/ml NS–398 (Fig 1 b). These results suggest that COX plays an important role in regulating RA synovial cell proliferation.

2. Effect of prostaglandin E\(_2\) on [\(^3\)H]–TdR incorporation into RA synovial fibroblasts

Cyclooxygenases are reported to play a critical role in PG synthesis in both physiological and pathological conditions.
Effect of Cyclooxygenase Inhibitor on Rheumatoid Synovial Fibroblasts Proliferation in Vitro

Fig. 1. Effect of COX inhibitors on RA synovial cell proliferation
The effect of COX inhibitors on RA synovial cell proliferation was detected as described in materials and methods. RA synovial fibroblasts were cultured in the presence of various concentrations of COX inhibitor, (a) resveratrol or (b) NS–398 for 72-hour and incorporation of [³H]-TdR were detected.

Prostaglandin E₂, which is reported to suppress the RA synovial cell proliferation in vitro, is one of the major metabolites in this pathway. It is, therefore, postulated that augmentation of [³H]-TdR incorporation into RA synovial fibroblasts by resveratrol may be mediated through the inhibition of PGE₂ synthesis by resveratrol. In order to confirm this hypothesis, the effect of PGE₂ on resveratrol–induced RA cell proliferation was investigated.

As shown in Fig. 2, 5 μg/ml of resveratrol increased [³H]-TdR incorporation into RA synovial fibroblasts in comparison with resveratrol untreated control. When PGE₂ was added into the synovial cell culture, it suppressed the cell proliferation with or without resveratrol in a dose dependent fashion and 10⁻¹² M of PGE₂ reversed the stimulation of cell proliferation by resveratrol to basal level.

These results revealed that COX–1 selective inhibitor, resveratrol stimulated cell proliferation by inhibiting PGE₂ generation in RA synovial fibroblasts.

Discussion

Rheumatoid arthritis is considered to be a chronic inflammatory disorder accompanied by both inflammatory cell infiltration and synovial cell proliferation. Pronounced synovial hyperplasia yields to pannus tissue which invades both articular cartilage and subchondral bone and deteriorates the joint function. Inhibition of pannus formation, therefore, would have the potential to slow or reduce joint destruction in rheumatoid arthritis since
pannus tissue may contribute the perpetuation of rheumatoid inflammation and the exacerbation of joint destruction due to secretion of various growth factors, cytokines, PGE2, matrix metalloproteinases, and nitric oxide.

PGE2 is detectable at a high level in the fluid of knee joints in OA\textsuperscript{12, 13} and RA\textsuperscript{14, 15}. PGs including PGE2 are synthesized from eicosatetraenoic acids in the presence of 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\textsuperscript{16}. 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\textsuperscript{17}. These results suggested that PGE2 might exacerbate joint inflammation and be involved in the disease process of degenerative arthritis.

Recently, the administration of NSAIDs is reported to reduce the risk of fatal colon cancer\textsuperscript{16}. Though it is still elusive why NSAIDs can prevent the carcinogenesis of colon, following observations could support these epidemiological reports. Enhanced expression of COX–2 polypeptides in human colon cancer tissues was detected using immunohistochemistry, whereas COX–1 expression was weak in both normal and cancerous specimens\textsuperscript{4}. It is also shown that overexpression of COX–2 leads to phenotypic changes involving the inhibition of apop-
Effect of Cyclooxygenase Inhibitor on Rheumatoid Synovial Fibroblasts Proliferation in Vitro

tosis in intestinal epithelial cells that could enhance their tumorigenic potential\(^\text{19}\). Thus, COX-2 may be strongly related to the cell proliferation including colon carcinogenesis.

On the other hand, PGE\(_2\) is reported to be an antiproliferative molecule and one of the inducers of apoptotic change in various types of cells. For instance, PGE\(_2\) induces cAMP accumulation and inhibits the growth of the most differentiated breast cancer cells due to loss and probably dysfunction of PGE\(_2\) receptors\(^\text{19}\). PGE\(_2\) also suppressed RA synovial cell proliferation through intracellular cAMP accumulation\(^\text{11}\). In terms of the apoptosis by PGE\(_2\), it is involved in apoptotic alterations typical of ovarian surface epithelium associated with ovulatory ovine follicles\(^\text{20}\) and induction of thymocyte apoptosis for negative selection of T lymphocytes\(^\text{20}\).

We have studied the effect of the COX inhibitors on RA synovial cell proliferation in this paper. The inhibition of COX-1 by resveratrol in RA synovial fibroblasts has resulted in the augmentation of cell proliferation, which is reversed to basal level by adding PGE\(_2\). Whereas, selective COX-2 inhibitor, NS-398 suppressed RA synovial cell proliferation. NS-398 is reported to abolish the PGE\(_2\) synthesis of human gingival fibroblasts induced by inflammatory cytokines\(^\text{22}\). It is still unknown why suppression of COX-2 activities may lead to antitumorigenic action. Detail studies should be employed to dissolve this divers effect of COX-2 and PGE\(_2\). Recently, selective COX-2 inhibitors are utilized as NSAIDs with less divers effects such as gastric ulcer or renal dysfunction, which might result from COX-1 inhibition by conventional NSAIDs such as indomethacin. Based on our findings, administration of selective COX-2 inhibitors may suppress pannus formation and prevent the progression of joint destruction in RA patients. Therefore, it is suggested that COX-2 selective NSAIDs may have chemopreventive effect on RA.

References

8. Sakurada S, Kato T, Okamoto T. Induction of cytokines and ICAM–1 by proinflammatory cytokines in primary rheumatoid synovial fibroblasts and inhibition by N–ace-


