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Expression of Cyclooxygenase-1 and -2 in Rheumatoid Arthritis Synovium

The aim of this study was to investigate the expression and localization of cyclooxygenase-1 and -2 (COX-1 and COX-2) in synovial tissues from patients with rheumatoid arthritis (RA). Synovial tissues from 9 patients with RA and 5 patients with osteoarthritis (OA) were examined for COX-1 and COX-2 expressions by immunohistochemical staining using 2 polyclonal COX-1 and COX-2 antibodies. In RA synovia, synovial lining cells showed intense immunostaining for COX-1, whereas slight to moderate staining was observed in inflammatory cells, stromal fibroblast-like cells and vascular endothelial cells. There was no significant difference in COX-1 expression between RA and OA synovia. The localization of COX-2 expression clearly differed from that of COX-1 expression, being most intense in inflammatory cells. However, there was no difference in COX-1 and COX-2 expressions between RA and OA synovial tissues. Our observations support that inflammatory mechanisms modulated by COX-1 and COX-2 in chronic RA synovium might be similar to those in chronic OA synovium.

Key Words : *Arthritis, Rheumatoid; Prostaglandin-Endoperoxide Synthase; Synovial Membrane*

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of unknown cause characterized by hyperplasia of synovial lining cells, infiltration of mononuclear cells into the synovial membrane, and extensive destruction of the articular cartilage (1). This abnormal proliferation of synovial cells is an important event in the pathophysiology of RA, as synovial proliferation leads to pannus formation, which can aggressively invade bone and cartilage (2). The occurrence of newly formed blood vessels (3), and pleomorphic fibroblast-like cells with large nuclei and prominent nucleoli (4) are also similar to preneoplastic conditions.

Cyclooxygenase (COX) is a key regulatory enzyme in the synthesis of prostaglandins (PG) which are important autocrine and paracrine mediators of inflammation in RA. COX exists in at least two isoforms, COX-1 and COX-2 (5, 6). COX-1 is constitutively expressed in most tissues and is thought to generate PG for physiologic functions, such as gastrointestinal protection, vascular homeostasis, and renal function (7). COX-2 is almost undetectable under physiologic conditions but up-regulation of COX-2 by cytokines or growth factors appears to produce PG mainly at sites of inflammation (8). Several studies have suggested that increased COX could play a role in synovial inflammation of RA (9-11).

However, until recently, studies on COX-1 and COX-2 expression in synovial tissues from RA showed various results (8, 12-20), and the expression and role of COX-1 and COX-2 in synovial tissue from RA remain to be more clarified. The aim of this study was to investigate the expression and localization of COX-1 and COX-2 in RA synovium.

MATERIALS AND METHODS

Patients

Synovial tissue specimens were obtained from nine patients with RA undergoing either synovectomy or joint replacement surgery. All patients included in this study satisfied the diagnostic criteria of the American College of Rheumatology (21). All patients were taking nonsteroidal antiinflammatory drugs (NSAIDs), four were treated with disease modifying anti-rheumatic drugs (DMARD), and four were receiving low dose prednisone.

Table 1. Clinical features of the patients

Patient	Age/Sex	Duration of disease (yrs)	RF (IU/mL)	Surgical procedure
RA				
1	46/M	10	648	THRA
2	56/F	16	52	Knee synovectomy
3	50/F	12	129	TKRA
4	47/F	5	92	Wrist synovectomy
5	63/F	5	34	TKRA
6	49/F	7	354	TKRA
7	42/F	13	143	TKRA
8	40/F	10	40	TKRA
9	51/F	13	192	THRA
OA				
1	68/M	2	-	TKRA
2	62/F	5	-	TKRA
3	64/F	6	-	TKRA
4	71/F	12	-	TKRA
5	54/F	10	-	TKRA

RA, rheumatoid arthritis; OA, osteoarthritis; RF, rheumatoid factor; THRA, total hip replacement arthropathy; TKRA, total knee replacement arthropathy

For comparison, synovial tissue samples were also obtained from patients with OA undergoing joint replacement surgery. The diagnosis of OA was based on typical clinical and radiological features. All patients were taking NSAIDs. Clinical characteristics of the patients were presented in Table 1. Tissue samples were fixed in 10% formalin at room temperature immediately after removal.

Immunohistochemistry

We purchased COX-1 and COX-2 antibodies which are affinity-purified goat polyclonal antibodies raised against a peptide corresponding to amino acids 570-599 and 583-604 mapping at the carboxy terminus of the human COX-1 and COX-2, respectively (Santa-Cruz Biotechnology, Santa-Cruz, CA, U.S.A.). The DACO LSAB plus kit, peroxidase (DACO A/S, Glostrup, Denmark) was used for immunohistochemistry.

Consecutive 5 μm thick paraffin-embedded sections were prepared using standard methods. All samples were stained with hematoxylin and eosin. Avidin-biotin complex (ABC) immunoperoxidase staining was performed using COX-2 polyclonal antibodies and DACO LSAB plus kit according to the manufacturer's protocol for paraffin sections. After deparaffinizing the sections, the sections were immersed for 30 min in 0.3% peroxide in methanol to deplete endogenous peroxidase. Nonspecific binding sites were saturated with 0.3% bovine serum albumin and normal goat serum for 30 min. Primary antibodies against COX-1 and COX-2 were used at dilution of 1:100 and applied to tissue sections and incubated in a humidified chamber at a room temperature for 2 hr. The sections were then washed with phosphate buffered saline (PBS). Biotinylated goat antibodies were applied onto the sections and incubated at room temperature for 20 min. After washing with PBS, the sections were incubated with streptavidin peroxidase. A chromogen of 3-amino-9-ethylcarbazole (AEC) was used to localize the secondary antibodies. Counterstaining was performed with Mayer's hematoxylin. Control staining on all synovial specimens was identical except that incubation with primary antibodies was not done. Positive staining was indicated as brown deposits. The extent and intensity of staining with COX-1 and COX-2 antibodies were graded on a scale of 0–4+ on two separate occasions using coded slides and an average score was calculated. A 4+ grade implies that all staining was maximally intense throughout the specimen, while 0 implies that staining was absent throughout the specimen.

Inflammation index

One section stained with hematoxylin and eosin was used for histologic features of inflammation. Six variables were assessed, based on the protocol described previously (22, 23), with the modification that a grading system of 0-3 was used. Grading was based on polymorphonuclear (PMN) cell infiltration, hyperemia, fibrin deposition, mononuclear cell infiltration, synovial proliferation, and fibrosis. The assessment was made on the whole specimen, both at low power and high power by two independent pathologists who were blinded to the clinical data.

Statistical analysis

Data were expressed as mean ± SEM. The statistical testing of histologic scores and staining patterns of COX-1 and COX-2 between RA and OA was done by chi-square test or contingency table analysis. Differences were considered to be significant when $p<0.05$. Statistical analysis was performed with SPSS for Windows 7.0 (SPSS Inc.).

RESULTS

In histologic appearance, PMN cell infiltration, fibrin deposition, mononuclear cell infiltration and synovial cell proliferation were observed in the synovial tissues from RA more than in those from OA ($p<0.05$). Fibrosis was seen in the synovial tissues from RA more than in those from OA, but this did not reach statistical significance ($p>0.05$). There was no difference in hyperemia between

Table 2. Results of histologic scoring

Patient	Polymorphonuclear* cell infiltration	Hyperemia	Fibrin* deposition	Mononuclear* cell infiltration	Synovial* cell proliferation	Fibrosis
RA						
1	1	1	1	1	2	2
2	2	1	2	2	1	0
3	1	2	1	1	1	0
4	1	1	2	2	2	1
5	1	1	1	1	0	1
6	3	1	1	2	2	1
7	1	2	0	2	0	2
8	1	2	1	1	1	2
9	1	2	1	2	1	2
Mean (SEM)	1.3 (0.2)	1.4 (0.2)	1.1 (0.2)	1.6 (0.2)	1.1 (0.3)	1.1 (0.2)
OA						
1	0	1	0	0	0	1
2	2	2	2	3	1	1
3	0	1	0	0	0	0
4	0	1	0	0	0	0
5	2	2	2	2	1	2
Mean (SEM)	0.8 (0.5)	1.4 (0.2)	0.8 (0.5)	1.0 (0.6)	0.6 (0.4)	0.8 (0.4)

RA, rheumatoid arthritis; OA, osteoarthritis; *$p<0.05$, compared with OA

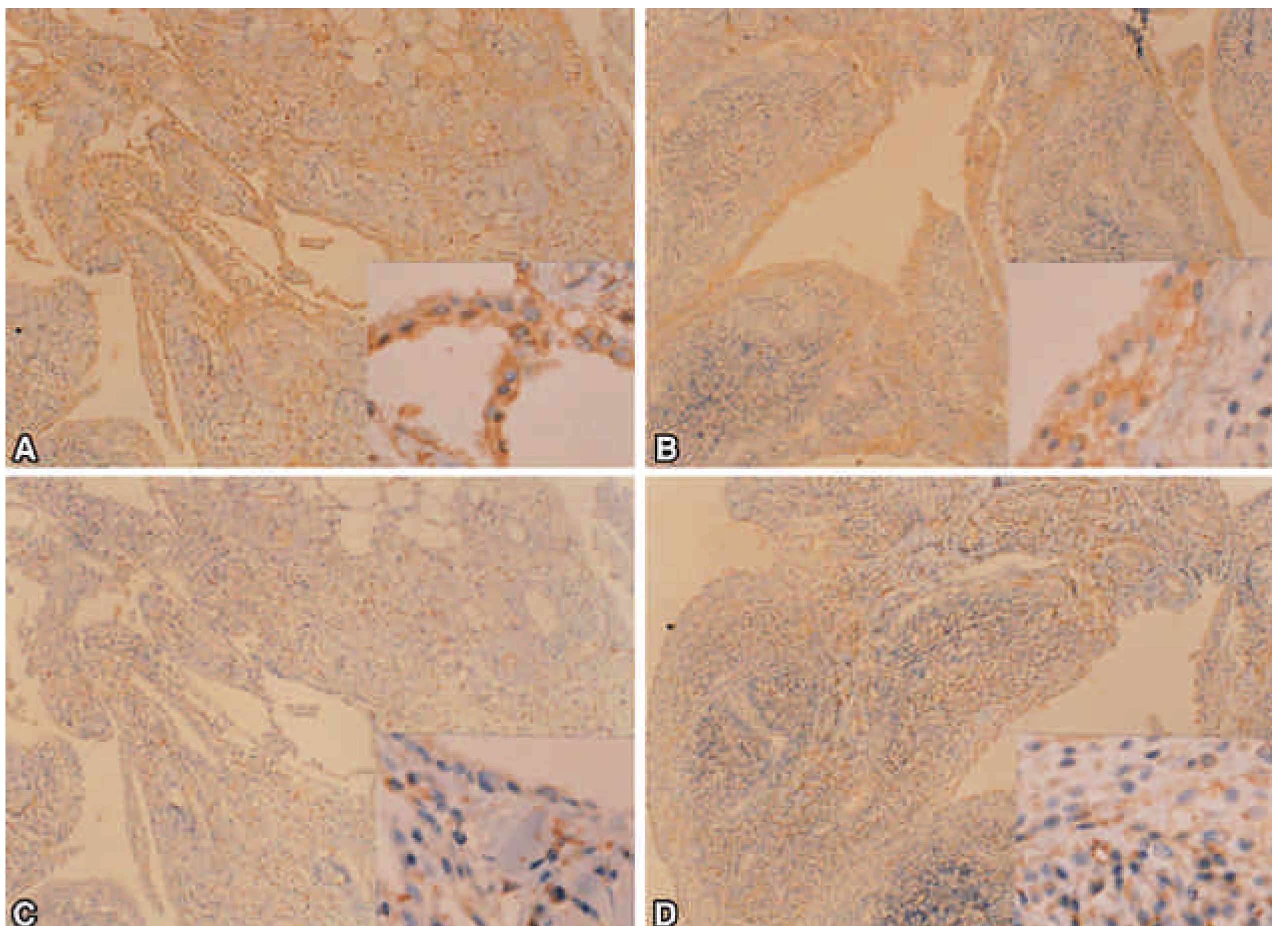

Fig. 1. Comparison of immunohistochemical stainings for COX-1 (A, B) and COX-2 (C, D) between the same synovial tissues from OA (A, C) and RA (B, D). Positive immunoreactivity appeared as brown. COX-1 expression was intense in synovial lining cells from OA (A) and RA (B) synovial tissues and COX-2 expression was intense in inflammatory cells from OA (C) and RA (D) synovial tissue (×100). Inset, high power field (×400) showing positive cells.

Table 3. COX-1 expression in synovial tissues from patients with RA and OA

	RA	OA
Synovial lining cells	3.1±0.1 (3 - 4+)	3.1±0.1 (3 - 4+)
Mononuclear inflammatory cells	1.9±0.2 (1 - 3+)	2.0±0.3 (1 - 3+)
Stromal fibroblast-like cells	1.9±0.2 (1 - 3+)	2.2±0.4 (1 - 3+)
Vascular endothelial cells	1.4±0.1 (1 - 2+)	1.4±0.2 (1 - 2+)

Graded 0 - 4+ on coded sections by two blinded observers
0, no staining; 4+, maximum intensity
Values are the mean±SEM (range)

Table 4. COX-2 expression in synovial tissues from patients with RA and OA

	RA	OA
Synovial lining cells	1.9±0.2 (1 - 2+)	1.4±0.2 (1 - 2+)
Mononuclear inflammatory cells	2.5±0.3 (2 - 3+)	1.8±0.5 (1 - 3+)
Stromal fibroblast-like cells	1.5±0.3 (1 - 2+)	1.4±0.2 (1 - 2+)
Vascular endothelial cells	1.3±0.3 (1 - 2+)	1.2±0.3 (0 - 2+)

Graded 0 - 4+ on coded sections by two blinded observers
0, no staining; 4+, maximum intensity
Values are the mean±SEM (range)

the two groups (Table 2).

COX-1 and COX-2 were readily detected in synovial tissues from RA and OA (Fig. 1).

COX-1 was expressed most strongly in synovial lining cells from RA synovial tissues and expressed in stromal fibroblast-like cells, inflammatory mononuclear cells and vascular endothelial cells. In OA synovial tissues, COX-1 expression was not different from that seen in RA synovial tissues (Table 3) (Fig. 1). COX-2 was stained most intensively in mononuclear inflammatory cells from RA synovial tissues unlike the intense immunostaining for COX-1 in synovial lining cells, and expressed in stromal fibroblast-like cells, synovial lining cells and vascular endothelial cells. However, there was no significant difference of COX-2 staining between synovial tissues from RA and OA (Table 4) (Fig. 1).

DISCUSSION

We studied the expression and localization of COX-1 and COX-2 in synovial tissue of RA and OA. Previous studies on COX expression in RA synovium (8, 17-20) have shown various results in site and extent of COX expression in RA synovium. Siegle et al. revealed that strong COX-2 immunostaining was observed in the endothelium of blood vessels in RA synovia, as well as in synovial lining cells, chondrocytes, and subsynovial fibroblast-like cells (20). In contrast to OA synovial tissues, COX-1 staining was almost exclusively localized in synovial lining cells with no significant differences between the two groups, and COX-2 mRNA expression was higher in RA than in OA samples (20). Crofford et al. revealed that COX-2 was expressed in infiltrating mononuclear cells, endothelial cells of blood vessels, and subsynovial fibroblast-like cells, but COX-2 was stained weakly in the synovial lining cell layer (8). Franz et al. showed that a distinct expression of COX-1 could be shown in RA and OA in fibroblast-like cells throughout the synovial lining and sublining layers. None of the cells of the microvasculature expressed COX-1 mRNA and the distribution of COX-2 expression resembled that of COX-1. Unlike other studies, they suggested that inflammatory mechanisms modulated by COX-1 and COX-2 in chronic RA synovium might be similar to those in chronic OA synovium (19).

In addition to various results of COX expression in RA synovium, recent studies suggest that COX-2 inhibition is not always beneficial for the gastrointestinal tract in contrast to previous findings (8). PGs derived from COX-2 can have important antiinflammatory effects and mucosal protection in experimental colitis, and COX-1 is responsible for a majority of enzyme activity in both the basal and stimulated states and can contribute to inflammation (24, 25). Also, no differences in clinical efficacy are evident between NSAIDs exhibiting preferential activity for either COX-1 or COX-2, and although those exhibiting a preferential action on COX-2 are generally less toxic, exceptions do exist (26). It has also been reported that gastrointestinal abnormalities do not develop in COX-1 knockout mice (27) and COX-2 knockout mice appear to have a normal response to inflammatory stimuli (28). Taken together, these findings suggest that COX-1 may play a role in inflammation. If this is true, an ideal balance of COX-2 and COX-1 inhibition from an NSAID can be important in inflammation control.

The results of our study showed that COX-1 expression was most intense in synovial lining cells from OA and RA synovial tissues and COX-2 expression was most intense in inflammatory cells from RA and OA synovial tissues. But there was no significant difference in COX-1 and COX-2 expressions between RA and OA synovial tissues. Our observations were similar to the results by Franz et al. (19).

In conclusion, we found that there was no difference in COX-1 and COX-2 expressions between RA and OA synovial tissues. Our observations support that inflammatory mechanisms modulated by COX-1 and COX-2 in chronic RA synovium might be similar to those in chronic OA synovium. In order to clarify the exact role for COX-1 and COX-2 in synovial inflammation of RA, further studies will be needed.

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