Effect of Antiinflammatory Drugs on COX-1 and COX-2 Activity in Human Articular Chondrocytes

FRANCISCO J. BLANCO, RAMÓN GUITIAN, JORGE MORENO, FRANCISCO J. de TORO, and FAUSTO GALDO

ABSTRACT. ***Objective.*** To study the effect of steroidal and nonsteroidal antiinflammatory drugs (NSAID) on cyclooxygenase (COX-1 and COX-2) activity in human articular chondrocytes.

Methods. Chondrocytes were isolated from articular cartilage of donors with no articular disease. Unstimulated and interleukin 1 (IL-1) stimulated chondrocytes were used as models to study the effects of drugs on COX-1 and COX-2. Cells were incubated with vehicle or drugs; supernatants were removed and the level of prostaglandin E_2 (PGE_2) in each sample was determined by enzyme immunoassay. IC50 were calculated from the reduction in PGE_2 content by different concentrations of the test substance by linear regression analysis.

Results. COX-1 mRNA was detected in unstimulated cells, but stimulation with IL-1 for up 12 h did not modify the levels of COX-1 mRNA. In contrast, COX-2 mRNA was not detectable in unstimulated cells, but it was induced by IL-1. Dexamethasone inhibited COX-2 mRNA expression induced by IL-1. COX-2 protein levels correlated with mRNA expression. Dexamethasone was the strongest drug inhibitor of COX-2 (IC50 = 0.0073 μM). However, it did not inhibit COX-1 activity. Among all NSAID tested, meloxicam and aspirin were the least potent inhibitors of COX-1 (IC50 = 36.6 μM and 3.57 μM, respectively). Indomethacin and diclofenac were the most potent inhibitors of COX-1 (IC50 = 0.063 μM and 0.611 μM, respectively) and COX-2 isoforms (IC50 = 0.48 μM and IC50 = 0.63 μM, respectively). Meloxicam was a more potent inhibitor of COX-2 (IC50 = 4.7 μM) than aspirin (IC50 = 29.3 μM) and similar to piroxicam (IC50 = 4.4 μM). Among all drugs tested dexamethasone showed the greatest selectivity for COX-2 and meloxicam was the NSAID with the best COX-2/COX-1 ratio (r = 0.12). Aspirin and piroxicam were about 8 times more active against COX-1 than COX-2, indomethacin was 7 times more active, and diclofenac was an equipotent inhibitor of COX-1 and COX-2.

Conclusion. We found that COX-1 and COX-2 isoforms are expressed in human chondrocytes at rest and in IL-1 stimulated cells, respectively. Antiinflammatory drugs have different capacities to inhibit COX enzyme in human articular chondrocytes. (J Rheumatol 1999;26:1366–73)

Key Indexing Terms:
CARTILAGE OSTEOARTHRITIS CHONDROCYTES
CYCLOOXYGENASE MELOXICAM

Cyclooxygenase (COX) is the first enzyme along the pathway in which arachidonic acid is converted to prostacyclin and prostaglandins. Two isoforms of COX are known to be present in eukaryotic organisms: COX-1 (constitutive) and COX-2 (inducible)[1-3]. The deduced amino acid sequence of hCOX-2 is 61% identical to the hCOX-1 protein and the expression of the isoenzymes shows some tissue-specific differences[4-7]. Under basal conditions COX-1 mRNA and protein are present in virtually all tissues and, in some of them, COX-2 mRNA and enzyme were also found at low levels[8,9]. However, COX-2 mRNA and protein levels are markedly increased after treatment with interleukin 1ß (IL-1ß) or phorbol-12-myristate-13-acetate (PMA); dexamethasone markedly suppresses the induction of COX-2 mRNA[4,10,11]. In sharp contrast, the transcription of COX-1 is not modulated by either IL-1ß or dexamethasone[12,13]. These results suggest that COX-1 is present in cells under physiological conditions, whereas COX-2 is induced by some cytokines, mitogens, and endotoxins, presumably in pathological conditions, such as inflammation[11,14-16].

Cartilage is the target for extracellular matrix destruction in inflammatory arthropathies and the primary site for pathogenic processes in osteoarthritis (OA). Chondrocytes are the only cell type in articular cartilage and a source, together with synovial tissue, of increased prostaglandin synthesis in arthritis[17,18]. Unstimulated human chondrocytes do not contain detectable COX-2 mRNA, but it is induced by IL-1ß[19]. However, in rabbit articular chondrocytes, COX-2 mRNA was constitutively expressed, and its presence

From the Laboratory of Cartilage Research, Rheumatology Unit and Tissue Bank, Juan Canalejo Hospital, A Coruña; Fundación Juan Canalejo, A Coruña; and Boehringer Ingelheim, Madrid, Spain.

Supported by grants from the University of A Coruña, Boehringer-Ingelheim-Madrid, and FIS-Spain, Expediente 98/1138.

F.J Blanco, MD, Staff; F.J. de Toro, MD, Staff; F. Galdo, MD, Chief of Rheumatology Service, Complejo Hospitalario Juan Canalejo; R. Guitian, Fundacion Juan Canalejo; J. Moreno, MD, Boehringer Ingelheim.

Address reprint requests to Dr. F.J. Blanco Garcia, Laboratory of Cartilage Research, Rheumatology Unit, Complejo Hospitalario Juan Canalejo, 15006 A Coruña, Spain. E-mail: fjblanco@ctv.es

Submitted March 11, 1998 revision accepted November 23, 1998.

increased in cultures treated with IL-1. Further, COX-1 mRNA was not detected in control or IL-1 treated cultures[20]. The role of prostaglandins in homeostasis of cartilage is unknown, and both anabolic and catabolic effects have been reported. Prostaglandin E_2 (PGE_2) plays a role in the regulation of chondrocyte proliferation and the synthesis of cellular matrix components[21-23]. High PGE_2 levels in the articular cartilage may inhibit cell proliferation[24,25].

COX inhibitors are used widely in the treatment of inflammatory arthropathies and represent the major pharmacological intervention in OA[26,27]. The effect of antiinflammatory drugs — both nonsteroidal and steroidal — in cartilage is controversial[27,28]. Inhibition of COX by drugs has been of great interest, in particular the question of which of the COX enzymes is inhibited[29,30]. Absolute IC50 values and IC50 ratio values of COX-2/COX-1 vary depending on the study, and they are influenced by the species and the models used (e.g., protein versus whole cells)[31]. The inhibitors of COX-2 are potent antiinflammatory agents that do not produce the typical side effects associated with the nonselective, COX-1 directed antiinflammatory agents[16,32]. IL-1 stimulated human articular chondrocytes and OA chondrocytes express high levels of COX-2 mRNA and protein[19,33].

We investigated the effects of antiinflammatory drugs on the activity of COX isoforms in human articular chondrocytes because (1) the expression and modulation of COX-1 and COX-2 vary according to the cellular species used; (2) the inhibitory selectivity of antiinflammatory drugs against the isoforms of COX is not similar in all species, tissues, and cells; (3) NSAID are the principal modality for treatment of inflammatory arthropathies and they represent the major pharmacological intervention in OA.

MATERIALS AND METHODS

Specimen selection. Cartilage tissue from 17 donors (9 men, 8 women, ages ranging from 45 to 64 years) with no history of joint disease was provided by the Tissue Bank and the Autopsy Service at Juan Canalejo Hospital.

Chondrocyte isolation. Cartilage slices were removed from the femoral condyles and washed in Dulbecco's modified Eagle's medium (DMEM). Slices were minced with a scalpel, transferred into a digestion buffer containing DMEM, 5% fetal bovine serum (FBS), 1% L-glutamine, penicillin 150 U/ml, streptomycin 50 mg/ml, and 2 mg/ml clostridial collagenase (Type IV, Sigma, St. Louis, MO, USA). Cartilage was incubated on a shaker at 37°C until the fragments were digested. Residual multicellular aggregates were removed by sedimentation (1 × g). The cells were filtered through nylon mesh with a pore diameter of 25 µm and were washed 3 times in RPMI 5% FBS, penicillin, and streptomycin before use. Isolated chondrocytes were not pooled and each experiment was carried out employing cells from the same donor.

Western blot for COX-1 and COX-2. Chondrocytes were plated in 6 well plates and stimulated for 24 h. Cells were washed with ice cold phosphate buffered saline (PBS) once. Cell pellets were lysed by boiling for 5 min in 20 µl of lysis buffer (1% sodium dodecyl sulfate, 10 mM Tris, pH 7.4) and microfuged for 5 min. The supernatants were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and the proteins transferred to nitrocellulose membranes. The blots were blocked with 5% bovine serum albumin (BSA) overnight at 4°C and incubated with rabbit anti-COX-1 polyclonal antibody or mouse anti-COX-2 monoclonal antibody (Cayman Chemical Co., California) at 1:1000 dilution for 1 h at room temperature. After 5 washes with Tris buffered saline (TBS)/Tween (125 mM NaCl, 25 mM Tris, pH 8.0, 0.1% Tween 20), the blots were incubated with horseradish peroxidase conjugated goat anti-rabbit IgG or anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) in TBS with 1% BSA for 1 h at room temperature. After a 30 min washing with TBS/Tween, the blots were incubated with ECL substrate solution (Amersham, New Jersey) for 1 min according to the manufacturer's instructions. After 30 to 60 min, the blots were exposed to radiographic film for 10 to 30 s.

Northern blot analysis. Total RNA was isolated with RNA STAT from 10^6 cells and 20 µg was tested per line in agarose-formaldehyde gel electrophoresis. The RNA was transferred to nylon filters and crosslinked by exposure to UV light for 5 min. The filters were prehybridized in 50% formamide, 6 × SSC, 0.5% SDS, 5 × Denhardt's solution, and 100 µg/ml yeast RNA for 4 h at 42°C. Overnight hybridization was performed at 42°C in the same buffer containing 10^6 cpm/ml probe followed by washes in 1 × SSC, 0.1% SDS at room temperature (2 × 30 min) and in 0.1 × SSC, 0.1% SDS at 60°C (2 × 30 min). The filters were exposed to Kodak XAR film at –70°C for 24 h. Polymerase chain reaction (PCR) was used to generate cDNA fragments specific for human COX-1 and COX-2 from reverse transcribed human chondrocyte total RNA using PCR primers[3]. The amplified PCR products were directly cloned into PCR TM II prokaryotic TA cloning system (Invitrogen, Carlsbad, CA, USA). A human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) cDNA fragment was purchased from Clontech (Palo Alto, CA, USA). These fragments were subsequently labeled by the random primer method (Boehringer-Mannheim Corp., Indianapolis, IN, USA).

Drug effects on COX-1 activity. Indomethacin, aspirin, piroxicam, diclofenac, dexamethasone (Sigma), and meloxicam (Boehringer Ingelheim, Ingelheim, Germany) were used at concentrations between 10 mol/l and 10^{-9} mol/l. In each experiment all drugs were tested using cells from the same donor. Three assays with duplicate wells were performed. Resting cells (not treated with IL-1) were used to study the effects on COX-1. Cells (10^6 cells/well in 6 well plates with 1.5 ml of DMEM and 5% FBS) were cultured and incubated with different concentrations of drugs for 30 min. Then 30 µM arachidonic acid was added for 15 min at 37°C. Supernatant was collected to determine PGE_2 levels.

Drug effects on COX-2 activity. IL-1 stimulated cells were employed to assay COX-2 activity. The contribution of chondrocyte COX-1 to total COX activity was suppressed by treating the cells with aspirin[34]. Cells (10^6 cells/well in 6 well plates with 1.5 ml of DMEM and 5% FBS) were cultured with aspirin (10 mol/l) for 12 h; then the media were removed and cells were washed 3 times with PBS. Chondrocytes were stimulated with IL-1 (5 ng/ml) for 24 h to induce synthesis of COX-2. Supernatant was collected to determine PGE_2 levels. Then drugs were added at different concentrations for 30 min; 30 µM arachidonic acid was added for 15 min at 37°C. Supernatant was collected to determine PGE_2 levels.

PGE_2 assay. The conditioned media from chondrocytes (cultured at 10^6 cells/well in 6 well plates in 1.5 ml media) were collected and stored at –80°C. PGE_2 content was measured by enzyme immunoassay (EIA) (Amersham) according to the manufacturer's instructions.

FACS analysis. Flow cytometric analysis was used to quantify COX-2 protein. The cells were plated in 6 well plates (500,000 cells/well), stimulated for 24 h, harvested, and fixed in 100 µl of 2% paraformaldehyde in PBS (pH 7.4). After 10 min at 4°C, 10 µl of 0.5% Triton X-100 (Sigma) in PBS was added. Ten minutes later, the cells were washed twice in PBS containing 1% BSA and then incubated 10 min with 10% goat serum. Washed cells were stained for 16 h at 4°C with monoclonal FITC conjugated antibody to human prostaglandin H synthase 2 (Cayman Chemical). Cells were washed 3 times in PBS and then analyzed on a FACScan flow

cytometer (Becton Dickinson). Normal (nonimmune) FITC conjugated antibody was used as negative control.

Data analysis. Results are presented as the mean of 3 individual experiments performed in duplicate wells (total = 6 samples per drug tested). All results are reported as percentage of enzymatic activity, mean of control PGE_2 levels ± standard error. IC50 was calculated from the reduction in PGE_2 content by different concentrations of the test substance by linear regression analysis. The estimated function employed was: y = b0 — b0/[1 + (x/b2)b1]. In this model y = response, b0 = expected response at saturation, b2 = concentration for a half-maximal response, b1 determines the shape of the function, and x = dose of drug. Correlation between 2 variables was determined by linear regression analysis.

RESULTS

Characterization of mRNA-COX isoforms in human articular chondrocytes. We examined *in vitro* articular human chondrocytes for COX-1 and COX-2 mRNA expression (Figure 1). COX-1 was detected in samples from unstimulated cells (Figure 1A, lane 1) and stimulation with IL-1 (1 ng/ml) for up to 12 h did not modify the COX-1 levels in these cells (Figure 1A, lane 2). However, chondrocytes stimulated with IL-1 for 6 h expressed COX-2 (Figure 1B, lane 2), which was not detected in cells at rest (Figure 1B, lane 1). We also found that dexamethasone (10 nM) completely inhibited IL-1 induced COX-2 mRNA expression (Figure 1B, lane 3), but did not affect the COX-1 mRNA level (Figure 1A, lane 3).

Characterization of enzyme-COX isoforms in human articular chondrocytes. Protein findings in chondrocytes showed results similar to mRNA expression (Figure 2). In cells at

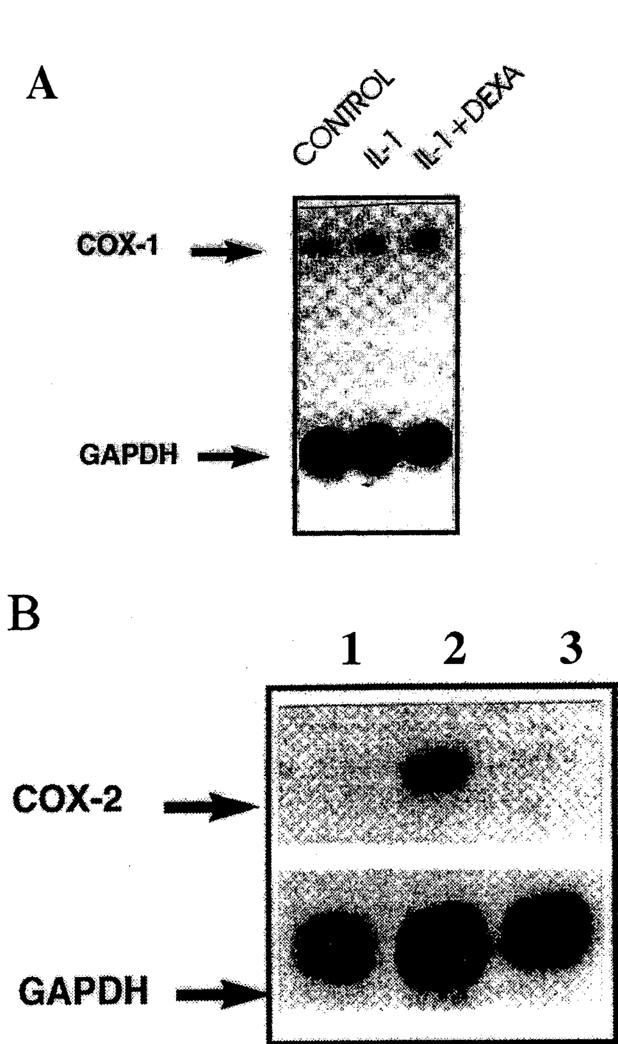


Figure 1. mRNA analysis by Northern blot of COX-1 and COX-2 expression in chondrocytes. A. Cells at rest contain COX-1 (lane 1); exposure to IL-1 (1 ng/ml) for 12 h and to IL-1+ dexamethasone (10 nM) did not modify COX-1 expression (lane 2 and 3). B. Cells at rest did not express COX-2 (lane 1); IL-1 (1 ng/ml) for 6 h stimulated COX-2 expression (lane 2), and dexamethasone (10 nM) totally inhibited effect of IL-1 on COX-2 expression (lane 3).

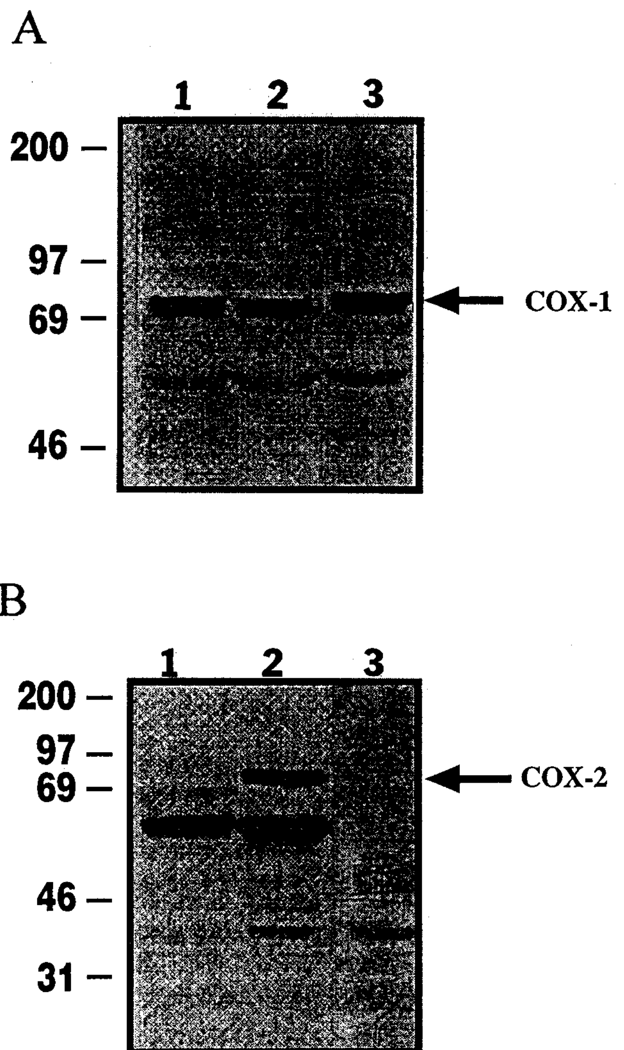


Figure 2. Western blot of COX-1 and COX-2 protein in chondrocytes: cell lysates were separated by 10% SDS-PAGE, transferred to nitrocellulose filter, and probed with antibodies to COX-1 and COX-2. A. Cell extracts of chondrocytes at rest (lane 1) contained a 70 kDa protein that was recognized by antibodies to COX-1. Exposure to IL-1 (1 ng/ml) for 24 h did not modify the expression of the protein (lane 2). B. Cell lysates of IL-1 (1 ng/ml) stimulated chondrocytes contained COX-2 (lane 2), but lysates from cells at rest did not express it (lane 1).

rest COX-1 antibody recognized a band of 70 kDa (Figure 2A, lane 1) that was not identified by COX-2 antibodies (Figure 2B, lane 1). IL-1 did not modify protein COX-1 expression (Figure 2A, lane 2). In contrast, COX-2 protein was present only in IL-1 treated human chondrocytes (Figure 2B, lane 2). Cells at rest and cells stimulated with IL-1 and dexamethasone did not show COX-2 expression.

Analysis of COX-1 and COX-2 activities in human articular chondrocytes. The above results suggest that resting cells express only COX-1, and IL-1 stimulated cells express both COX-1 and COX-2. Although IL-1 did not modify COX-1 expression, we precultured the cells with aspirin before adding IL-1 to inhibit mainly COX-1 activity[33]. The final results are cells that express only the active isoform of COX-2, because residual COX-1 activity was suppressed with aspirin. As an indicator of COX enzyme activity we measured PGE_2 levels in supernatants. The sensitivity of PGE_2 EIA was 17 pg/ml. To avoid individual variability we used cells from the same donor to test all antiinflammatory drugs. The basal release of PGE_2 in unstimulated chondrocytes was 130 ± 12 pg/ml and 2174 ± 97 pg/ml in IL-1 (1 ng/ml) stimulated chondrocytes after 24 h. To analyze the correlation between COX-2 expression and PGE_2 levels, cells from the same donor were stimulated with IL-1 at concentrations between 0.01 and 5 ng/ml. We found that the synthesis of PGE_2 by the IL-1 stimulated cells correlated with COX-2 protein expression quantified by FACS (Table 1 and Figure 3). Based on these findings we used cells at rest and IL-1 stimulated chondrocytes as models to analyze the effect of antiinflammatory drugs on COX-1 and COX-2 activity, respectively.

Table 1. COX-2 expression and PGE_2 levels. Chondrocytes were stimulated with the indicated concentrations of stimuli and inhibitors. Conditioned media were collected after 24 h for PGE_2 EIA, and the cells were harvested for flow cytometry.

Conditions	COX-2, %	PGE_2, pg/ml, mean ± SE
Control	< 1	130 ± 12
IL-1, 1 ng/ml	87	2174 ± 97
TNF, 5 ng/ml	68	1451 ± 63
IL-1 + DEX, 100 nM	12	215 ± 54

DEX: dexamethasone.

Effect of steroids and NSAID on complete human articular chondrocytes. Dexamethasone, a potent steroid, was the strongest drug inhibitor of COX-2 (IC50 = 0.0073 μM). Dexamethasone at a concentration of 10^{-3} M totally inhibited the COX-2 activity. However, it did not inhibit COX-1 activity (Table 2 and Figure 4).

Among all NSAID tested, meloxicam and aspirin were the least potent inhibitors of COX-1 (IC50 = 36.6 μM and 3.57 μM, respectively). Indomethacin and diclofenac were the most potent inhibitors of both COX-1 (IC50 = 0.063 and 0.611 μM) and COX-2 isoforms (IC50 = 0.48 and 0.63 μM). Meloxicam was a more potent inhibitor of COX-2 (IC50 = 4.7 μM) than aspirin (IC50 = 29.3 μM) and similar to piroxicam (IC50 = 4.4 μM).

Among all drugs tested dexamethasone showed the greatest selectivity for COX-2, and meloxicam was the NSAID with the best COX-2/COX-1 ratio (r = 0.12). Aspirin and piroxicam were about 8 times more active against COX-1 than COX-2, and indomethacin was 7 times

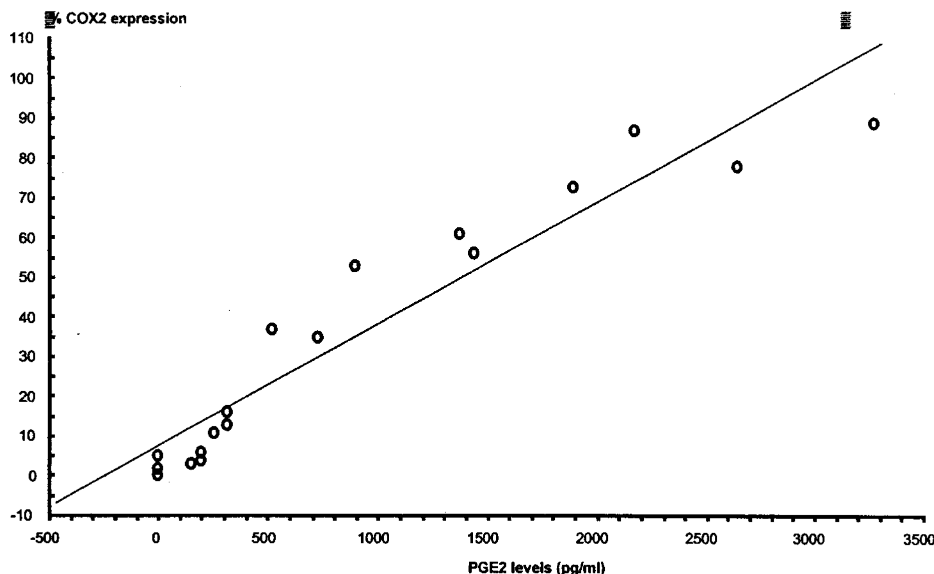


Figure 3. Correlation analysis of COX-2 expression by FACS and PGE_2 levels by EIA. Cells from the same donor were plated (10^6 cells/well) and stimulated with IL-1 at concentrations between 0.01 and 5 ng/ml. PGE_2 levels were quantified in the supernant and COX-2 expression was quantified by FACS. The synthesis of PGE_2 by the IL-1 stimulated cells correlates with COX-2 protein expression (r = 0.95067; p = 0.003519).

Table 2. IC50 values for NSAID effects on COX-1 and COX-2 activity in intact human articular chondrocytes. Data show the mean ± SE for 6 determinations calculated from the means of duplicate determinations. Ratios of IC50 values for the NSAID on COX-2 relative to COX-1 are given in the last column.

NSAID	COX-2, mol/l	COX-1, mol/l	Ratio COX-2/COX-1 (n = 6)
Aspirin	0.0293 ± 0.00634	0.00357 ± 0.000579	8.20
Indomethacin	0.000483 ± 0.0000517	0.0000630 ± 0.00000365	7.66
Piroxicam	0.0353 ± 0.00283	0.00441 ± 0.000611	8.01
Diclofenac	0.000634 ± 0.0000334	0.000603 ± 0.0000497	1.03
Meloxicam	0.00471 ± 0.000763	0.0366 ± 0.00351	0.12

more active. Finally, diclofenac was an equipotent inhibitor of COX-1 and COX-2.

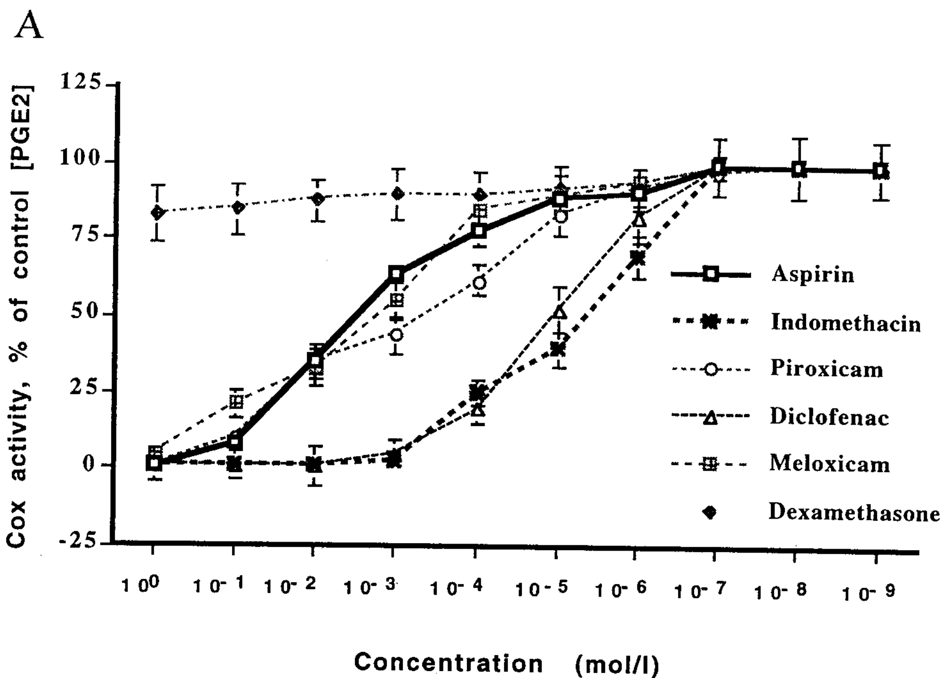
DISCUSSION

Two forms of cyclooxygenase, COX-1 and COX-2, are known to be present in eukaryotic organisms[1-3]. Although the expression of the isoenzymes shows some cell-specific differences, under basal conditions mostly COX-1 is present and COX-2 is markedly increased by treatment with IL-1ß, lipopolysaccharide, or PMA[4,6,9-11]. Dexamethasone markedly suppresses the induction of COX-2 mRNA[10,35]. In contrast, COX-1 transcripts are not modulated by IL-1ß or dexamethasone[12,13]. Based on these results it has been proposed that COX-2 is responsible for the synthesis of inflammatory prostaglandins, whereas COX-1 is a "housekeeping" enzyme, producing physiologically relevant prostanoids[11,14-16,36]. Recently, it was reported that unstimulated human chondrocytes did not express detectable COX-2 mRNA, but this was induced by IL-1ß[19]. Expression and modulation of COX-1 was not analyzed in this study. We found that COX-1 mRNA and protein are present in chondrocytes at rest, and that IL-1 did not modify COX-1 expression. These results are concordant with findings in bovine chondrocytes and human osteoblasts[37,38]. However, they are different from findings in rabbit chondrocytes[20].

Different methods have been used to study the effects of NSAID on COX-1 and COX-2 activity *in vitro*[16,29,39-41]. These include purified enzyme systems (in microsomal membranes from Cos-1 cells transfected with human or murine isoenzymes), cultured cells selectively expressing COX-1 or COX-2, and cultured intact cells. It has been reported that intact cells isolated from normal tissues that selectively express COX-1 or COX-2 provide a more suitable screening system for selective inhibitors[16,30,41]. As the inhibition test with different drugs was measured by means of PGE_2 production, and to obtain a model that expresses only COX-2, we decided to preincubate the cells with aspirin to inhibit mainly COX-1 activity (and probably also COX-2, but resting human chondrocytes did not express significant levels of COX-2). In this manner, all PGE_2 measured after IL-1 stimulation is produced by a COX-2 isoform synthesized *de novo*. Aspirin showed no effect on the COX-1 and COX-2 proteins or on mRNA expression (data not shown)[34]. Further, we have proved that the PGE_2 level measured in the supernatant correlated with COX-2 protein concentration quantified by FACS. Based on these findings we selected resting cells and IL-1 stimulated cells as models to study the inhibitory activity of antiinflammatory drugs against COX-1 and COX-2, and we calculated their IC50 values and IC50 ratio in relation to COX-2/COX-1.

Using this model we found differences in the way some antiinflammatory drugs inhibit the COX-1/2 isoforms. Dexamethasone, an antiinflammatory steroid, was the drug with the greatest selectivity for COX-2. The second best was meloxicam, with a COX-1/2 ratio of 0.12. However, classical NSAID such as aspirin, piroxicam, and indomethacin inhibited mainly COX-1. This result is similar to findings relating to human synovial cells. Recently, it has been reported that dexamethasone has a similar effect on bovine chondrocytes[38]. The authors compared dexamethasone with NS398, a selective inhibitor of COX-2, and they found no marked differences regarding the effect on cyclooxygenase activity.

Cartilage homeostasis involves synthesis of the macromolecules of the extracellular matrix (proteoglycan and collagen), and regulation of enzymes that degrade the extracellular matrix, such as metalloproteases. Chondrocytes produce PGE_2, prostacyclin, and prostaglandin F_2, and the synthesis of these prostanoids by cartilage can be increased by biologically relevant factors such as IL-1, tumor necrosis factor-α, and trauma[42,43]. IL-1 induces degradation of human cartilage explants and some findings suggest that PGE_2 may mediate the IL-1 effect on cartilage degradation[44]. However, prostaglandins may also have positive effects on cartilage, through increasing the level of glucocorticoid receptors in chondrocytes, influencing cartilage differentiation and proliferation, and mediating the effects of vitamin D on cartilage[45]. The effects of NSAID on chondrocytes and in cartilage are controversial. There is a strong body of evidence to suggest that NSAID do exert beneficial effects on cartilage, and there is also evidence that some NSAID accelerate cartilage damage *in vitro* in both animal and human models[27,28,46]. Experiments using aged cartilage from femoral heads indicate that indomethacin and sodium



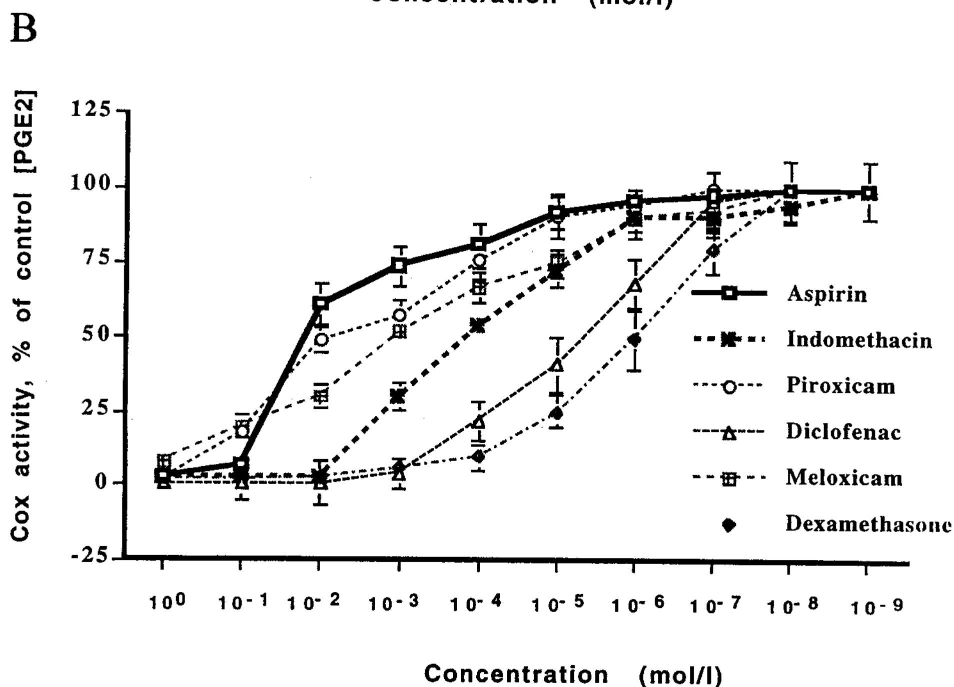


Figure 4. The effect of dexamethasone, aspirin, indomethacin, piroxicam, diclofenac, and meloxicam on (A) COX-1 (resting chondrocytes) and (B) COX-2 (IL-1 stimulated chondrocytes). COX activity was measured by the formation of PGE_2 after exposure to exogenous arachidonic acid 30 μM for 15 min. Dexamethasone was the strongest drug inhibitor of COX-2. However, it did not inhibit COX-1 activity. Indomethacin, aspirin, and piroxicam were more potent inhibitors of PGE_2 formation from COX-1 cells than from COX-2 chondrocytes. Diclofenac was an equipotent inhibitor and meloxicam was a more potent inhibitor of COX-2 than COX-1. Data are expressed as mean ± SE from 6 determinations from at least 3 separate experiments.

salicylates suppress chondrocyte proteoglycan synthesis[47]. Whether the inhibitory selectivity of NSAID on COX isoforms may explain the different effects of these drugs in human articular cartilage is not yet known.

The efficacy of nonsteroidal selective COX-2 inhibitors in chronic inflammatory disorders has yet to be determined. Theoretical and experimental considerations suggest that specific inhibitors of COX-2 are likely to have a more favor-

able side effect profile than the NSAID currently in use that inhibit both COX-1 and COX-2[16,32,48]. It has been reported that COX-2 is induced in cartilage and synovial tissues from patients with OA or RA[7,13,33]. Thus, it is conceivable that NSAID that selectively inhibit COX-2 activity in cartilage and synovial tissue may exhibit better therapeutic effects and fewer side effects in these rheumatic diseases. Of all NSAID tested in this *in vitro* study, meloxicam was the drug that most selectively inhibited COX-2 enzyme activity. Further, *in vitro* studies performed on human synovial cells showed similar results to those performed on human chondrocytes[49].

The full range of a particular NSAID's effects depends in part on the cartilage cells' origin[50]. For example, in human primary cultures, naproxen and diclofenac had no significant effects on glycosaminoglycan (GAG) synthesis or cell proliferation, even at high doses, but GAG synthesis was stimulated by ibuprofen at 10 µg/ml[51]. However, diclofenac sodium, indomethacin, piroxicam, naproxen, ibuprofen, and salicylic acid inhibit GAG synthesis in high concentrations in secondary cultures of rabbit chondrocytes[52]. The effect of a particular NSAID on proteoglycan synthesis in normal cartilage also differs from its effect in OA cartilage. *In vivo* and *in vitro* studies indicate that some NSAID may reduce the degradation of human OA cartilage. Therapeutic concentrations of tiaprofenic acid significantly reduce proteoglycan catabolism by decreasing metalloprotease synthesis. The effect of tiaprofenic acid on cartilage metabolism is, almost partially, mediated through the inhibition of COX[50]. In contrast, studies on normal animal cartilage have shown that NSAID have no significant effect on cartilage catabolism. An explanation of the discrepancy between rabbit and human articular chondrocytes and between healthy human chondrocytes and OA chondrocytes could be due to the different profiles of COX-1 and COX-2 expression and modulation that appear in human and in rabbit cells[20], and to the different levels of COX-2 in healthy and OA cartilage[33].

This study has some limitations. All NSAID tested in this study were capable of inhibiting the COX-1 isoenzyme. The analysis comparing their IC50 with their concentrations in plasma and synovial fluid of patients treated with these drugs shows that meloxicam, aspirin, and indomethacin have IC50 values for COX-1 similar to their concentrations in plasma. In contrast, the IC50 values of piroxicam and diclofenac for COX-1 are higher than the plasma levels. Similar results were found in relation to COX-2. Furthermore, we used cells and not cartilage tissue. Perhaps a study in the natural environment of chondrocytes would identify the potency of antiinflammatory drugs on cartilage, and these experiments would also show the ability of antiinflammatory drugs to penetrate the cartilage. This approach is being explored in our laboratory. Another limitation is that it is not possible to exclude the participation of other enzymes normally implicated in the arachidonic acid cascade, such as phospholipase A_2, from the results obtained with PGE_2 production, and the influence of IL-1 at other points of the arachidonic acid cascade is also impossible to regulate.

In conclusion, we found that both COX-1 and COX-2 isoforms are expressed in human chondrocytes at rest and IL-1 stimulated cells, respectively. *In vitro* research with human chondrocytes yields valuable information on the bioactivity profiles of drugs on chondrocyte metabolism. Antiinflammatory drugs have different capacities to inhibit the COX enzyme in human articular chondrocytes. Indomethacin, aspirin, and piroxicam principally inhibit COX-1, diclofenac is equipotent, meloxicam inhibits COX-2 preferentially, and dexamethasone does not inhibit COX-1.

ACKNOWLEDGMENT

The authors express appreciation to Dr. Pilar Sánchez for her expert technical support in the FACS analysis and to the Department of Orthopedics, Department of Pathology, and the Tissue Bank of the Complejo Juan Canalejo for providing cartilage samples.

REFERENCES

1. DeWitt D, Smith W. Primary structure of prostaglandin G/H synthase from sheep vesicular gland determined from the complementary sequence. Proc Natl Acad Sci USA 1988;85:1412-6.
2. Xie W, Chipman J, Robertson D, Erikson R, Simmons D. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proc Natl Acad Sci USA 1991;88:2692-6.
3. Hla T, Neilson K. Human cyclooxygenase-2 cDNA. Proc Natl Acad Sci USA 1992;89:7384-92.
4. O'Banion M, Winn V, Young D. cDNA cloning and functional activity of a glucocorticoid-regulated inflammatory cyclooxygenase. Proc Natl Acad Sci USA 1992;89:4888-92.
5. Jones D, Carlton D, McIntyre T, Zimmerman G, Prescott S. Molecular cloning of human prostaglandin endoperoxide synthase type II and demonstration of expression in response to cytokines. J Biol Chem 1993;268:9049-54.
6. O'Neill G, Ford-Hutchinson A. Expression of mRNA for cyclooxygenase-1 and cyclooxygenase-2 in human tissues. FEBS Lett 1993;330:156-60.
7. Crofford L. Cox-1 and Cox-2 tissue expression: Implications and predictions. J Rheumatol 1997;24 Suppl 49:15-9.
8. Davi G, Averna M, Catalano I, et al. Increased thromboxane biosynthesis in type IIa hypercholesterolemia. Circulation 1992;85:1792-8.
9. Seibert K, Zhang Y, Leahy K. Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. Proc Natl Acad Sci USA 1994;91:12013-7.
10. Lee SH, Soyoola E, Chanmugam P, et al. Selective expression of mitogen-inducible cyclooxygenase in macrophages stimulated with lipopolysaccharide. J Biol Chem 1992;267:25934-8.
11. Hempel S, Monick M, Hunninghake G. Lipopolysaccharide induces prostaglandin H synthase-2 protein and mRNA in human alveolar macrophages and blood monocytes. J Clin Invest 1994;93:391-6.
12. Smith W, DeWitt D. Biochemistry of prostaglandin endoperoxide H synthase-1 and synthase-2 and their differential susceptibility to nonsteroidal antiinflammatory drugs. Semin Nephrol 1995; 15:179-94.
13. Crofford L, Wilder R, Ristimaki A, et al. Cyclooxygenase-1 and -2

expression in rheumatoid synovial tissues. Effects of interleukin-1ß, phorbol ester, and corticosteroids. J Clin Invest 1994;93:1095-101.
14. Vane JR, Mitchell JA, Appleton I, et al. Inducible isoforms of cyclooxygenase and nitric-oxide synthase in inflammation. Proc Natl Acad Sci USA 1994;91:2046-50.
15. Ristimaki A, Garfinkel S, Wessendorf J, Macing T, Hla T. Induction of cyclooxygenase-2 by interleukin-1. J Biol Chem 1994; 269:11769-75.
16. Masferrer J, Zweifel B, Manning P, et al. Selective inhibition of inducible cyclooxygenase-2 in vivo is antiinflammatory and nonulcerogenic. Proc Natl Acad Sci USA 1994;91:3228-32.
17. Wittenberg R, Willburger R, Kleemeyer K, Peskar B. In vitro release of prostaglandins and leukotrienes from synovial tissue, cartilage and bone in degenerative joint diseases. Arthritis Rheum 1993;36:1444-50.
18. Salmon J, Higgs G, Vane J, et al. Synthesis of arachidonate cyclooxygenase products by rheumatoid and nonrheumatoid synovial lining in nonproliferative organ culture. Ann Rheum Dis 1983;42:36-9.
19. Geng Y, Blanco FJ, Cornelisson M, Lotz M. Regulation of cyclooxygenase-2 expression in normal human articular chondrocytes. J Immunol 1995;155:796-801.
20. Lyons-Giordano B, Pratta M, Galbraith W, Davis G, Arner A. Interleukin-1 beta differentially modulates chondrocyte expression of cyclooxygenase-2 and phospholipase A_2. Exp Cell Res 1993;206:58-64.
21. Blanco F, Lotz F. IL-1 induced nitric oxide inhibits chondrocyte proliferation via PGE_2. Exp Cell Res 1995;318:319-25.
22. Dingle JT. Cartilage maintenance in osteoarthritis: interaction of cytokines, NSAID, and prostaglandins in articular cartilage damage and repair. J Rheumatol 1991;18 Suppl 28:30-7.
23. O'Keefe R, Crabb I, Puzas J, Rosier R. Influence of prostaglandins on DNA and matrix synthesis in growth plate chondrocytes. J Bone Miner Res 1992;7:397-405.
24. Steinberg JJ, Sledge CB. Chondrocyte mediated cartilage degradation: regulation by prostaglandin E_2, cAMP and interferon α. J Rheumatol 1991;18 Suppl 27:63-5.
25. Verbruggen G, Veys E. Regulation of chondrocytes in rheumatoid arthritis. In: Adolphe M, editor. Biological regulation of the chondrocytes. Boca Raton: CRC Press; 1992:275-94.
26. Hochberg H, Altman R, Brandt K, et al. Guidelines for the medical management of osteoarthritis. Arthritis Rheum 1995;38:1541-6.
27. Brandt K. Effects of nonsteroidal anti-inflammatory drugs on chondrocyte metabolism in vitro and in vivo. Am J Med 1987;83 Suppl 5A:29-34.
28. Pelletier J, Haraoui B, Pelletier J. Modulation of cartilage degradation in arthritic diseases by therapeutic agents. In: Woessner H, Howell DS, editors. Joint cartilage degradation: Basic and clinical aspects. New York: Dekker; 1993.
29. Meade EA, Smith WL, DeWitt DL. Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other nonsteroidal antiinflammatory drugs. J Biol Chem 1993;268:6610-4.
30. Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ, Vane JR. Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proc Natl Acad Sci USA 1993;90:11693-7.
31. Pairet M, Engelhardt G. Differential inhibition of COX-1 and COX-2 in vitro and pharmacological profile in vivo of NSAID. In: Vane J, editor. Improved nonsteroid antiinflammatory drugs. London: William Harvey Press; 1996:103-20.
32. Vane JR, Botting RM. A better understanding of antiinflammatory drugs based on isoforms of cyclooxygenase (COX-1 and COX-2). Adv Prostaglandin Thromboxane Leukot Res 1995;23:41-8.
33. Amin A, Attur M, Patel R, et al. Superinduction of cyclooxygenase-2 activity in human osteoarthritis-affected cartilage. J Clin Invest 1997;99:1231-7.
34. Patrono C, Ciabattoni G, Patrignami P. Clinical pharmacology of platelet cyclooxygenase inhibition. Circulation 1985;72:1177-84.
35. Masferrer J, Seibert K, Zweifel B, Needleman P. Endogenous glucocorticoids regulate an inducible cyclooxygenase enzyme. Proc Natl Acad Sci USA 1992;89:3917-21.
36. Vane J. Towards a better aspirin [news comment]. Nature 1994;367:215-6.
37. De Brum-Fernandes A, Laporte S, Heroux M, et al. Expression of prostaglandin endoperoxide synthase-1 and prostaglandin endoperoxide-2 in human osteoblast. Biochem Biophys Res Commun 1994;198:955-60.
38. Morisset S, Patry C, Lora M, de Brum-Fernandes AJ. Regulation of cyclooxygenase-2 expression in bovine chondrocytes in culture by interleukin 1α, tumor necrosis factor-α, glucocorticoids, and 17ß-estradiol. J Rheumatol 1998;25:1146-53.
39. Mitchell MD, Edwin SS, Lundin-Schiller S, Silver RM, Smotkin D, Trautman MS. Mechanism of interleukin-1ß stimulation of human amnion prostaglandin biosynthesis: mediation via a novel inducible cyclooxygenase. Placenta 1993;14:615-25.
40. Gierse JK, Hauser SD, Creely DP, et al. Expression and selective inhibition of the constitutive and inducible forms of human cyclooxygenase. Biochem J 1995;305:479-84.
41. Leneaville O, Brener D, Dewitt D, Hla T, Funk C, Smith W. Differential inhibition of human prostaglandin endoperoxide H synthase-1 and -2 by nonsteroidal antiinflamatory drugs. J Pharmacol Exp Ther 1994;271:927-34.
42. Campbell I, Picolli D, Hamilton J. Stimulation of human chondrocyte prostaglandin E_2 production by recombinant human interleukin-1 and tumor necrosis factor. Biochem Biophys Acta 1990;1051:310-8.
43. Malemud C, Moskowitz R, Hassid A. Prostaglandin biosynthesis by lapine articular chondrocytes in culture. Biochem Biophys Acta 1981;663:480-90.
44. Chin J, Lin Y. Effects of recombinant human interleukin-1 beta on rabbit articular chondrocytes. Arthritis Rheum 1988;31:1290-6.
45. DiBattista J, Martel-Pelletier J, Cloutier J, Pelletier J. Modulation of glucocorticoid receptor expression in human articular chondrocytes by cAMP and prostaglandin. J Rheumatol 1991;17:102-5.
46. Rashad S, Revell P, Hemingway L. Effect of nonsteroid antiinflammatory drugs on the course of osteoarthritis. Lancet 1989;2:519-22.
47. McKenzie L, Horsburgh B, Ghosh P. Effect of antiinflammatory drugs on sulphated glycosaminoglycan synthesis in aged human articular cartilage. Ann Rheum Dis 1976;35:487-97.
48. Anderson G, Hauser S, McGarity K, Bremer M, Isakson P, Gregory S. Selective inhibition of cyclooxygenase (COX)-2 reverses inflammation and expression of COX-2 and interleukin 6 in rat adjuvant arthritis. J Clin Invest 1996;97:2672-9.
49. Vergne P, Bertin P, Liagre B, Bonnet C, Pairet M, Treves R. Differential inhibition of COX-1 and COX-2 by nonsteroidal antiinflammatory drugs in cultured synovial cells [abstract]. Arthritis Rheum 1997;40 Suppl:S93.
50. Pelletier JP, Cloutier JM, Martel-Pelletier J. In vitro effects of tiaprofenic acid, sodium salicylate and hydrocortisone on the proteoglycan metabolism of human OA cartilage. J Rheumatol 1989;16:646-55.
51. Smith RL, Kajiyama G, Lane NE. Nonsteroidal antiinflammatory drugs: effects on normal and interleukin 1 treated human articular chondrocyte metabolism in vitro. J Rheumatol 1995;22:1130-7.
52. Bjelle A, Eronen I. The in vitro effect of six NSAIDs on the glycosaminoglycan metabolism of rabbit chondrocytes. Clin Exp Rheumatol 1991;9:369-74.