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A R Amin, … , I R Patel, S B Abramson

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Research Article

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Superinduction of Cyclooxygenase-2 Activity in Human Osteoarthritis-affected Cartilage

Influence of Nitric Oxide

Ashok R. Amin,*§‖¶ Mukundan Attur,* Rajesh N. Patel,* Geeta D. Thakker,* Paul J. Marshall, John Rediske,** Steven A. Stuchin,‡ Indravadan R. Patel,* and Steven B. Abramson*§**

**Department of Rheumatology, ‡Department of Orthopedic Surgery, Hospital for Joint Diseases, New York 10003; §Department of Medicine, ‖Department of Pathology, ¶Kaplan Cancer Research Center, New York University Medical Center, New York 10016; and **Ciba-Geigy Corporation, Summit, New Jersey 07901*

Abstract

Cartilage specimens from osteoarthritis (OA)-affected patients spontaneously released PGE_2 at 48 h in ex vivo culture at levels at least 50-fold higher than in normal cartilage and 18-fold higher than in normal cartilage + cytokines + endotoxin. The superinduction of PGE_2 production coincides with the upregulation of cyclooxygenase-2 (COX-2) in OA-affected cartilage. Production of both nitric oxide (NO) and PGE_2 by OA cartilage explants is regulated at the level of transcription and translation. Dexamethasone inhibited only the spontaneously released PGE_2 production, and not NO, in OA-affected cartilage. The NO synthase inhibitor HN^G-monomethyl-L-arginine monoacetate inhibited OA cartilage NO production by > 90%, but augmented significantly (twofold) the spontaneous production of PGE_2 in the same explants. Similarly, addition of exogenous NO donors to OA cartilage significantly inhibited PGE_2 production. Cytokine + endotoxin stimulation of OA explants increased PGE_2 production above the spontaneous release. Addition of L-NMMA further augmented cytokine-induced PGE_2 production by at least fourfold. Inhibition of PGE_2 by COX-2 inhibitors (dexamethasone or indomethacin) or addition of exogenous PGE_2 did not significantly affect the spontaneous NO production. These data indicate that human OA-affected cartilage in ex vivo conditions shows (*a*) superinduction of PGE_2 due to upregulation of COX-2, and (*b*) spontaneous release of NO that acts as an autacoid to attenuate the production of the COX-2 products such as PGE_2. These studies, together with others, also suggest that PGE_2 may be differentially regulated in normal and OA-affected chondrocytes. (*J. Clin. Invest.* 1997. 99:1231–1237.) Key words: nitric oxide • osteoarthritis • cyclooxygenase • cytokine • cartilage

Introduction

Osteoarthritis (OA)[1] is a disease that affects the weight-bearing joints and peripheral and axial articulations. The clinical correlates are pain and restricted motion (1), and although the disease is classified as a noninflammatory arthropathy, several inflammatory components have been implicated in the disease process and have been observed in the synovial fluid. These include inflammatory cytokines, immunoglobulins, and other mediators in the joints (2, 3). Increased production of acute phase reactants such as transferrin, ceruloplasmin, acid glycoprotein, and increased concentrations of substance P have also been reported in OA (4). One of the hallmarks of OA is the progressive degeneration of the cartilage, where the synthetic and degradative forces are operating in tandem. Unchecked, the process leads to an imbalance of these forces and joint destruction.

Prostaglandins are produced at elevated levels in inflamed tissues including rheumatoid synovium (5, 6). PGE_1 and PGE_2 contribute to synovial inflammation by increasing local blood flow and potentiating the effects of mediators such as bradykinin and IL-1β that induce vasopermeability (7). PGE_2 has also been shown to trigger osteoclastic bone resorption (8), suggesting that this molecule may contribute to the pathophysiology of joint erosion in RA.

The human articular chondrocytes, when stimulated with cytokines and/or endotoxin in vitro, release various inflammatory mediators, including nitric oxide (NO) and PGE_2 (9, 10). We (11) and others (12) have recently observed that OA-affected cartilage in ex vivo conditions spontaneously releases NO in quantities sufficient to cause cartilage damage. The importance of these findings is supported by experiments conducted in animal models of arthritis, where inhibitors of NO synthase (NOS) (13, 14) or cyclooxygenase (COX)-2 (15) can independently repress joint inflammation, concomitant with the attenuation of PGE_2 or NO synthesis. In the present study, we report that OA-affected cartilage (but not normal cartilage): (*a*) spontaneously releases PGE_2 and NO, which are coordinately regulated; and (*b*) shows upregulated COX-2 mRNA and COX-2 protein, which spontaneously releases substantial amounts of PGE_2 in ex vivo conditions. Furthermore, the NO produced by the OA-affected cartilage inhibits the autocrine (or cytokine + endotoxin-induced) PGE_2 production, thus indicating a negative effect of NO on PGE_2 synthesis.

Address correspondence to Ashok R. Amin, Department of Rheumatology, Room 1600, Hospital for Joint Diseases, 301 E. 17th Street, New York, NY 10003. Phone: 212-598-6537; FAX: 212-598-6168.

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1. *Abbreviations used in this paper:* COX-2, cyclooxygenase-2; L-NMMA, HN^G-monomethyl-L-arginine monoacetate; NF, nuclear factor; NO, nitric oxide; NOS, NO synthase; OA, osteoarthritis; PDTC, pyrrolidine dithiocarbamate; RT, reverse transcription.

Methods

Reagents and cell lines. A macrophage-like cell line, RAW 264.7, was obtained from American Type Culture Collection (Rockville, MD). Monoclonal α-mouse macrophage COX-2 antibody, which also cross-reacts with human COX-2, was obtained from Transduction Laboratories (Lexington, KY). Protease inhibitors, dexamethasone, cycloheximide, indomethacin, pyrrolidine dithiocarbamate (PDTC), aminoguanidine, sodium nitroprusside, and LPS were obtained from Sigma Chemical Co. (St. Louis, MO); human IL-1β and TNF-α were from Fisher Scientific Co. (Pittsburgh, PA); and H^{G}-monomethyl-L-arginine monoacetate (L-NMMA) from $Cyclo_3$pss BioChemical Corp. (Salt Lake City, UT).

Procurement of human cartilage. Cartilage slices were taken from the knees of patients with the diagnosis of advanced OA (age: 50–70 yr) who were undergoing knee replacement surgery, and from nonarthritic knees (normal controls: age 20–40 yr). The OA patients were free of steroidal/nonsteroidal antiinflammatory drugs for at least 2 wk before surgery. Nonarthritic knee cartilage was obtained from patients with fractures or from accident victims after knee amputation the same day.

Extraction of human cartilage COX-2 and Western blotting. Slices from articular cartilage were frozen at −70°C, milled to fine particulate in liquid nitrogen, and sequentially extracted (10 ml/g wet wt tissue) with neutral salt buffer (Tris HCl:saline) containing protease inhibitors (11). Samples were run on 10% SDS-PAGE gels under nonreducing conditions, transferred to nitrocellulose, and Western blotted with α-COX-2 antibody. Bound antibody was detected by a secondary antibody conjugated with horseradish peroxidase, and developed using the enhanced chemiluminescence Western blotting system (Amersham Corp., Arlington Heights, IL) on Kodak Xomatic x-ray film.

Determination of nitrite/PGE_2. NO production was measured by estimating the stable NO metabolite, nitrite, in conditioned medium by modified Griess reaction (16). PGE_2 (detection limit: 50 pg/ml) was determined in the culture supernatant using RIA Kit (Sigma Chemical Co.) according to the manufacturer's instructions.

RNA extraction from articular chondrocytes. The method we followed is basically as described by Adams et al. (17). The cartilage was milled into fine powder in liquid nitrogen and extracted with 4 M guanidium thiocyanate, 25 mM sodium citrate, 0.5% sodium dodecyl sarcosine, and 0.1 M 2-mercaptoethanol for 4 h on a rocker. It was then extracted with H_2O-saturated phenol followed with phenol/chloroform. The aqueous layer was layered onto cesium trifluoroacetate gradient for ultracentrifugation (24,000 rpm/24 h). The RNA pellet was dissolved in GuSCN and precipitated with alcohol in the presence of acetic acid. The RNA obtained with this method is pure enough for reverse transcriptase (RT)–PCR analysis.

RT-PCR analysis, cloning and sequencing. The presence of mRNA was analyzed by RT of total RNA (1 μg) followed by PCR amplification of the cDNA. The PCR was carried out in an automated DNA thermal cycle (Perkin-Elmer Cetus Instruments, Norwalk, CT). The cDNA from mRNA was prepared using a SuperScript™ RNase H Reverse Transcriptase (Gibco BRL, Gaithersburg, MD) and PCR amplification was performed in 50 μl solution containing 1.5 mM $MgCl_2$, 0.4 μM of each primer, 0.2 mM of each dNTP, 2.0 units of Taq DNA polymerase (Promega Corp., Madison, WI). The cycle conditions were 45 s at 94°C, 45 s at 60°C, and 2 min at 72°C for 20–30 cycles for both COX-2 and β-actin. The PCR product was analyzed by electrophoresis on 1.5% agarose gels. Verification that the amplified PCR product originated from RNA rather than DNA was accomplished by preparing duplicate RNA samples both untreated and treated with reverse transcriptase, followed by PCR amplification. The amplified PCR products were directly cloned into PCR™ II Prokaryotic TA Cloning System (Invitrogen, San Diego, CA), and then the sequence of clones was determined using M13 primers by the dideoxy chain termination method using an automated laser fluorescent DNA sequencer (ALF; Pharmacia Diagnostics, Piscataway, NJ).

Statistical analysis. The data represent a minimum of two experiments performed from separate normal individuals or OA patients. All experiments using cartilage slices were performed in triplicate or quadruplicate for each parameter studied. The *P* value was calculated using Student's *t* test between the parameters in the same experiment.

Results

Previous studies have shown that stimulation of articular cartilage leads to synthesis of (in order of decreasing quantity) PGE_2, $PGF_2\alpha$, PGI_2, and TXA_2 (18). A number of synovial cytokines, including IL-1β and TNF-α, or endotoxins (LPS) enhance PGE_2 and NO synthesis in cultured chondrocytes (9, 10). In the present study, we first examined whether cartilage slices from normal human cartilage responded in a similar fashion. Incubation of normal human cartilage slices with IL-1β + TNF-α + LPS caused a significant augmentation in PGE_2 and induction of NO production within 48 h in culture (Fig. 1). These experiments were consistent with previous observations of basal levels of PGE_2 released by normal chondrocytes (unlike NO production) that could be augmented with LPS or IL-1β (10). These experiments demonstrate that the isolated chondrocytes and those embedded in cartilage in vitro behaved similarly with respect to the simultaneous stimulation of NO and PGE_2 production. We next examined whether PGE_2 was also spontaneously released by the OA-affected cartilage in ex vivo conditions, as we have previously reported for NO (11). Indeed, all the OA-affected cartilage (samples from 10 different patients) spontaneously released both NO (44–265 μM) and PGE_2 (50–670 ng/ml per g tissue) in ex vivo conditions at 48 h (Fig. 2). Each of the samples tested, as expected, had high levels of NO spontaneously released in the culture medium, as previously observed (11). It should be noted that the lowest amount of PGE_2 spontaneously released (Fig. 2, patient 2, ∼ 50 ng/ml) was at least 10-fold higher than that produced by normal cartilage (Figs. 1 and 2) or spontaneously released by equivalent amounts of normal chondrocytes (10). Next, using various inhibitors, we examined whether the spontaneous production of PGE_2 by OA-affected cartilage was due to endogenous upregulation of the inducible COX-2. The release of both these mediators (NO and PGE_2) was sensitive to nuclear-factor (NF)–κB transcription factor inhibitor

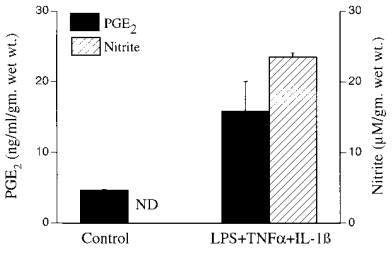


Figure 1. Modulation of NO and PGE_2 production in normal cartilage organ cultures. Normal knee articular cartilage from one individual was cut into 3-mm disks, and four to six disks were placed in organ cultures (in triplicate) of 2 ml media in the presence and absence of IL-1β (1 ng/ml) + TNF-α (100 U/ml) + LPS (100 μg/ml). The levels of nitrite and PGE_2 were monitored at 48 h as described in Methods. Data are expressed as micromolar of nitrite or nanograms per milliliter of PGE_2 per gram (wet wt) of cartilage. The *P* values based on the triplicate data ($n = 3$) between control (unstimulated) and cytokine + endotoxin-treated cultures were < 0.001 for PGE_2 production and < 0.0001 for nitrite accumulation. ND, not detectable (< 0.1 μM nitrite). Data represent one of three similar experiments.

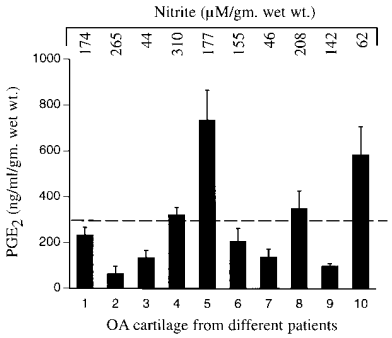


Figure 2. Spontaneous release of PGE_2 from OA-affected cartilage. Knee articular cartilage from 10 patients undergoing knee replacement surgery was set up in organ culture in triplicate ($n = 3$) as described in Fig. 1, without any modulators. Levels of nitrite/PGE_2 were monitored after 48 h, as shown in upper panel. The dotted line represents the mean amount of PGE_2 released by these 10 patients, which was 285 ng/ml per g tissue. Data are expressed as micromolar of nitrite or nanograms per milliliter of PGE_2 per gram (wet wt) of cartilage accumulated in the same medium at 48 h.

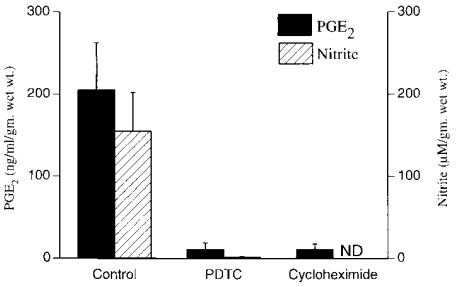
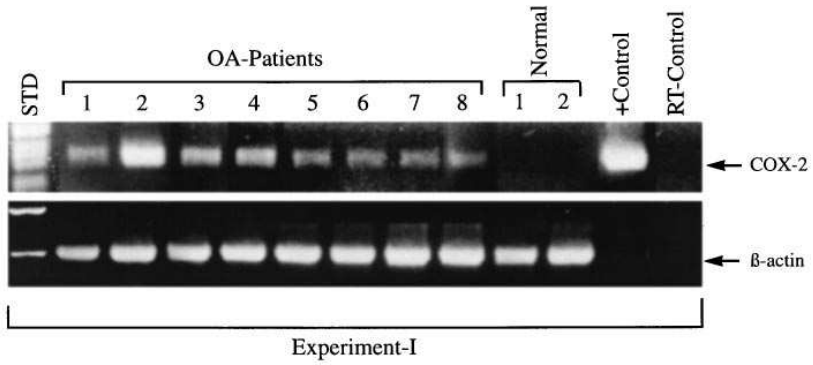


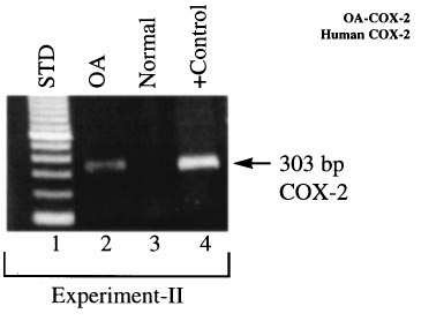
Figure 3. Comparison of NO and PGE_2 spontaneously produced by OA-affected cartilage. OA-affected knee articular cartilage from one patient was cut into 3-mm disks, and four to six disks were placed in organ cultures (in triplicate) in 2 ml of F-12 medium in the presence and absence of PDTC (20 μM) or cycloheximide (2 μg/ml). The levels of nitrite and PGE_2 were monitored after 48 h. Data are expressed as micromolar of nitrite or nanograms per milliliter of PGE_2 per gram (wet wt) of cartilage; $n = 3$. The P values between control (spontaneous release) versus PDTC-treated (nitrite < 0.0024; $PGE_2 < 0.0024$) or cycloheximide-treated cultures (nitrite < 0.0001; $PGE_2 < 0.0011$) were calculated. ND, not detectable (< 0.1 μM nitrite). The data represent one of two similar studies.

PDTC and translation inhibitor cycloheximide (Fig. 3), indicating the inducible nature of these mediators.

Since recent studies have implicated NF-κB as one of the essential transcription factors for the inducible COX-2 transcription (19), we examined the expression of inducible COX-2 mRNA directly from cartilage obtained from normal and OA-affected patients. Fig. 4, *A* and *B*, shows that all nine OA-affected cartilage samples tested showed upregulated COX-2 mRNA that could not be detected in the three normal cartilage samples tested, where the β-actin signal could be seen with the same mRNA preparations (not shown for Experi-



A



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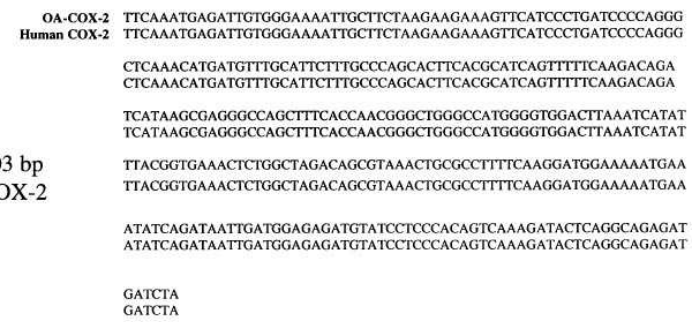

C

Figure 4. Expression of COX-2 and β-actin mRNA in normal and OA-affected cartilage. (*A*) Normal and OA-affected cartilage from different individuals was extracted for total RNA immediately upon receiving the samples without releasing the cells, as described in Methods. Equal amounts (100 ng) of total RNA were subjected to RT-PCR analysis (30 cycles) using specific human COX-2 primers (5′TTCAAATGAGATTGTG GGAAAAT 3′ and 5′AGATC ATCTCTGCCTGAGTATC-TT 3′) which generated a 303-bp fragment (20). The same RNA preparation (100 ng) was also subjected to RT-PCR analysis of β-actin using the primers 5′GTGGGGCGCCCCAGGC ACCA 3′ and 5′CTCCTTAAT-GTCACGCACGATTTC 3′ which generated a 475-bp fragment. The PCR primers designated here span one exon and two introns (20). STD designates mol wt standards. In one of the experiments (Experiment I), RT-PCR analysis of RNA from patient 4 was carried out without the reverse transcriptase enzyme (*RT:Control*), thus indicating the lack of potential DNA contamination. +Control designates amplification of COX-2 from a standard COX-2 cDNA. (*B* and *C*) The 303-bp COX-2 fragment amplified from the RNA of patient 9 (as shown in Experiment II) was cloned into a TA-cloning vector (ClonTech, Palo Alto, CA) and sequenced; it is compared above with the human COX-2 sequence (M90100).

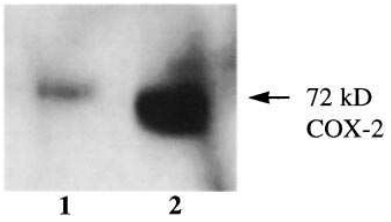

Figure 5. Western blot analysis of COX-2 from OA-affected cartilage. RAW 264.7 cells were stimulated with LPS to induce COX-2 as previously described (21). The cell-free extract was loaded on the SDS gel as shown in lane *1*. Total cartilage extract from the OA-affected cartilage was made as described in Methods and loaded onto the gel as shown in lane *2*. It was then Western blotted and probed with an anti-COX-2 mAb that binds to both murine and human COX-2, giving a signal at 72 kD.

ment II). The identity of the COX-2 mRNA was confirmed by Southern blot analyses of the 303-bp COX-2 fragments using a full-length COX-2 cDNA (data not shown) and sequencing one of the fragments from COX-2 mRNA amplified from OA-affected cartilage, as shown in Fig. 4, *B* and *C*. The partial DNA sequence of the OA-COX-2 obtained from one of the OA patients (*Experiment II*) showed > 98% homology with the known human COX-2 sequence (20). The expression of COX-2 was confirmed by Western blotting of COX-2 protein from OA-affected cartilage extracts which showed the expression of a 72-kD COX-2 protein as observed in LPS-stimulated macrophages (21) (Fig. 5). These experiments indicate that OA-affected cartilage spontaneously releases an inflammatory compound, PGE_2, in levels at least 50-fold higher (on average) than the normal cartilage, and this effect may be due to transcriptional upregulation of COX-2, which is sensitive to PDTC and cycloheximide.

We next examined the effect of endogenous NO on PGE_2 production in OA-affected cartilage. OA-affected cartilage slices were incubated in ex vivo conditions and the levels of spontaneously released NO and PGE_2 were monitored at 48 h in the presence and absence of the competitive NOS inhibitor L-NMMA (Table I). Addition of L-NMMA blocked > 90% of spontaneous endogenous NO production in these OA-affected cartilage organ cultures, as previously reported (11). However, the decrease in the NO production caused a significant augmentation (approximately twofold) of the PGE_2 accumulation in the same experiments. These experiments indicate that the levels of endogenous NO spontaneously released in ex vivo conditions inhibit the spontaneous production of PGE_2 in the same OA-affected cartilage slices. The effects of endogenous NO in OA cartilage on PGE_2 production could be mimicked by exogenously added NO donors such as sodium nitroprusside. In one representative experiment where OA-affected cartilage had released 221.7±70.2 ng/ml of PGE_2 (per g wet wt cartilage) at 72 h, addition of 100 μM of sodium nitroprusside (at 0 h) in the same explants significantly reduced PGE_2 production to 43.3±14.7 ($P < 0.004$; $n = 3$).

We then examined the effects of COX-2 products on NO production in OA explants as shown in Fig. 6. Addition of dexamethasone or indomethacin, as expected, inhibited (> 90%) the PGE_2 production in the OA-affected cartilage but had no significant effect on the levels of NO released by the same cartilage samples. Furthermore, as expected, addition of a COX-2 inhibitor, CGP28238 (Flosulide; Ciba-Geigy Corp., Summit, NJ) at 5 μM inhibited spontaneous PGE_2 production by OA-affected cartilage in ex vivo conditions by > 95% (like indomethacin), but had no significant effect on NO production (data not shown). We also tested the effects of exogenous PGE_2 on the spontaneous accumulation of nitrite in ex vivo conditions in our OA-explant assay. The OA-affected cartilage on an average released 28 ng/ml of PGE_2/100 mg cartilage as shown in Fig. 2. We therefore added 25 or 50 ng/ml of PGE_2 to the above OA cartilage explant assay. There was no significant effect on the accumulation of nitrite in OA-explants supplemented with 25 ng/ml PGE_2 (5.9±2.7 μM nitrite at 72 h;

Table I. Regulation of Spontaneously Released PGE_2 in OA-affected Chondrocytes by Endogenous NO

OA cartilage (treatment)	PGE_2	Nitrite
	ng/ml per g wt tissue	*μM/g wet wt tissue*
Experiment 1		
Control +	45.4±8.1	95.0±2.8
L-NMMA (500 μM)	115.7±55.0*	6.0±3.0*
Experiment 2		
Control +	205.0±58.0	155.3±47.0
L-NMMA (500 μM)	386.0±157.0*	8.2±3.0*
Experiment 3		
Control +	136.9±35.5	46.5±7.6
L-NMMA (500 μM)	187.5±42.0‡	1.0±0.1*
Experiment 4		
Control +	347.0±79.4	207.8±105.0
L-NMMA (500 μM)	786.2±112.0*	53.5±12.2*

Knee articular cartilage from four separate OA-affected patients was individually placed in organ culture in triplicate ($n = 3$) as described in Fig. 1. The release of NO/PGE_2 in the medium was monitored after 48 h in the presence and absence of 500 μM of L-NMMA. Data are expressed as micromolar of nitrite or nanograms per millilter of PGE_2 per gram (wet wt) of cartilage. The *P* value between the control (spontaneously released) and L-NMMA–treated cultures was within *< 0.005, ‡< 0.07 for each individual experiment.

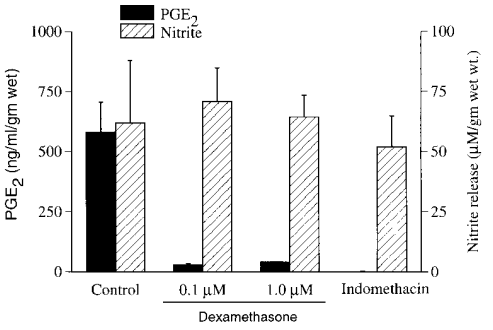

Figure 6. Regulation of PGE_2 production and NO in OA-affected cartilage by dexamethasone and indomethacin. Knee articular cartilage from an OA-affected patient was individually placed in organ culture (in triplicate) as described in Fig. 1. The release of NO/PGE_2 in the medium was monitored after 48 h in the presence and absence of dexamethasone (0.1–1 μM) and indomethacin (5 μM). Data are represented as micromolar of nitrite or nanograms per milliliter of PGE_2 per gram (wet wt) of cartilage. *P* values between the control and dexamethasone-treated cultures were: nitrite < 0.27, PGE_2 < 0.0001. *P* values between the control and indomethacin-treated cultures were: nitrite < 0.26, PGE_2 < 0.0001 ($n = 3$). The data represent one of two similar experiments.

Table II. Regulation of COX-2 by Intracellular NO in Cytokine + Endotoxin-stimulated OA-affected Cartilage in Ex Vivo Conditions

OA Cartilage (treatment)	PGE_2	Nitrite
	ng/ml per g wet wt tissue	*μM/g wet wt tissue*
Control	205.0±58.0*	155.3±47.0‡
L-NMMA (500 μM)	386.0±157.0*	8.2±3.0
PDTC (30 μM)	11.0±8.0	1.4±1.2
LPS + TNF-α + IL-1β	466.0±31.0*	240.7±36.9‡
LPS + TNF-α + IL-1β + L-NMMA	1838.0±349.0*	27.3±4.6
LPS + TNF-α + IL-1β + PDTC	14.8±3.2	4.7±4.2

Cartilage from one OA-affected patient was incubated in organ culture in triplicate ($n = 3$) as described in Fig. 1 and treated with cytokines + endotoxin, PDTC, or L-NMMA for 48 h. The levels of PGE_2 and nitrite were estimated from the same medium. The data represent one of two similar experiments; $n = 3$. P values: *< 0.005, ‡< 0.01.

$P < 0.30$, $n = 3$) or 50 ng/ml PGE_2 (4.2±3.3 μM nitrite at 72 h; $P < 0.21$, $n = 3$) as compared to control (2.8±0.6 μM at 72 h). These experiments demonstrate that in OA-affected cartilage, unlike in rat mesangial cells (22), inhibitors of PGE_2 synthesis or addition of exogenous PGE_2 had no significant effect on NO production.

We also examined the influence of cytokines and endotoxin on COX-2 expression and the effect of endogenous NO in OA-affected cartilage. As shown in Table II, production of both NO and PGE_2 in OA-affected cartilage is augmented significantly (1.6- and 2.3-fold, respectively) after exposure to LPS + TNFα + IL-1β. The inhibition of the NO production with L-NMMA under these conditions caused a dramatic (fourfold) increment in the PGE_2 accumulation, which exceeded that observed in the nonstimulated explants (Table II). As expected, PDTC blocked both the spontaneous and the cytokine + endotoxin-induced release of NO/PGE_2. These experiments indicate that the decreased levels of endogenous NO (in the presence and absence of cytokines + endotoxins + L-NMMA) in OA-affected cartilage cause a significant increase in PGE_2 production, thus again indicating that the endogenous NO released by OA-affected cartilage inhibits PGE_2 production, and could therefore act as a negative modulator of PGE_2 synthesis in OA-affected cartilage.

Discussion

These experiments raise the question: osteoarthritis or osteoarthrosis? Our data indicate that the spontaneous production of PGE_2 by human OA-affected cartilage is strikingly above that produced by normal cartilage. For example, 1 g of normal human cartilage releases ~ 5 ng/ml of PGE_2 at 48 h in ex vivo conditions described in this study. Addition of cytokines + endotoxin augments this effect to ~ 15 ng/ml. However, equivalent amounts of OA-affected cartilage in ex vivo conditions (and induced in vivo) show superinduction of PGE_2 production by releasing an average of ~ 285 ng/ml/g tissue (50-fold greater than the spontaneous release and 18-fold greater than the cytokine + endotoxin-induced release). PGE_2 is known to exacerbate joint inflammation (18) and induce IL-1β expression (23). Our experiments suggest that these inflammatory mediators (NO and PGE_2) may be involved in the disease process of OA. In rheumatoid disease, NO and PGE_2 could be expected to exert complex effects on chondrocytes as well as on the variety of cells involved in local and systemic immunological processes. In OA, on the other hand, these inflammatory mediators are likely to act in a more limited manner, exerting autocrine effects upon the metabolism of articular chondrocytes. For NO, the literature suggests that catabolic effects predominate, including the inhibition of proteoglycan and collagen synthesis as well as activation of metalloproteases (11, 12). For PGE_2, the net effects on chondrocytes are less clear. The quantity of PGE_2 produced could promote cartilage damage by exacerbating joint inflammation (18) and inducing IL-1β expression (23). However, exogenous PGE_2 added to rat chondrocytes stimulates [^{3}H]thymidine incorporation and aggrecan synthesis (24). In human OA-affected cartilage, exogenous PGE_2 upregulates glucocorticoid receptors (25). These latter two studies suggest potential beneficial or reparative effects of PGE_2 on matrix homeostasis in cartilage.

The elucidation of the role of COX-2 expression in a variety of other tissues may also be relevant to an understanding of its role in OA. Recent studies by Tsujii and DuBois (26) have shown that overexpression of COX-2 in rat intestinal epithelial cells causes increased adhesion to extracellular matrix proteins, lack of E-cadherin expression, reduced expression of $TGF\beta_2$ receptors, and resistance to butyrate-induced apoptosis with increased BCL-2 expression. Furthermore, additional evidence that the role of PGE_2 goes beyond that of a mere inflammatory mediator was obtained in experiments with COX-2 knockout mice, which showed high incidence of renal dysplasia, cardiac fibrosis, and infertility (in female mice) (27). Therefore, in view of the impact of PGE_2 levels (in transgenic and knockout systems) on various cellular functions, a reevaluation of its role in the current understanding of OA is warranted.

One of the most intriguing aspects of this study is that a pleiotropic molecule such as NO regulates another multifunctional mediator, PGE_2. Several studies in the literature indicate that NO (in the presence or absence of cytokines) enhances cyclooxygenase activity and PGE_2 production in various cell types including normal human chondrocytes (28–33). Salvemini et al. (34) have shown that injection of iNOS inhibitors into a rodent air pouch model (carrageenan-induced) blocked both iNOS and COX-2 activity in areas of inflammation. Our studies, in contrast, are similar to the studies by Stadler et al. (35) and Swierkosz et al. (36) that indicate that endogenous NO inhibits the synthesis of COX products as observed in rodent Kupffer cells and macrophages, respectively. This controversy may be due to the tissue-specific expression of NOS/COX-2 in various cell types, the difference in the pathophysiology of normal and OA-affected chondrocytes, and the influence of various other mediators in the microenvironment of the cartilage that may influence the production of NO and PGE_2. This is well demonstrated by Janabi et al. (37), where endogenous NO activated $PGF_2\alpha$ production in human microglial cells but not in astrocytes. Analysis of interactions between PGE_2 and NO showed that PGE_2 does not induce NO and is not required for NO induction by IL-1β (33). This latter observation is similar to those seen in this study. However, in contrast to studies

with OA-affected cartilage, exposure of normal chondrocytes to NO donors induced high levels of PGE_2, and NOS inhibitors (NMA) reduced the IL-1β–induced PGE_2 production.

Our studies demonstrate for the first time the negative influence of NO on PGE_2 synthesis in human OA-affected cartilage in ex vivo conditions. Similarities in the induction of these two mediators are of interest. Both NOS and COX-2 are upregulated in OA cartilage. In vitro, both enzymes are induced in chondrocytes by proinflammatory cytokines such as IL-1 and TNF-α. The production of both NO and PGE_2 by OA cartilage explants can be attenuated by inhibitors of protein synthesis (cycloheximide) and NF-κB (PDTC). An interesting divergence in the regulation of COX-2 and NOS in chondrocytes, however, is reflected in their sensitivity to glucocorticoids: in OA chondrocytes expression of COX-2, but not NOS, is inhibited by prior exposure to dexamethasone (11, 38).

Overall, these observations indicate that PGE_2 in chondrocytes plays a significant physiological role in cartilage homeostasis. These studies, together with our recent observation that nonsteroidal antiinflammatory drugs such as aspirin (but not indomethacin or sodium salicylate) can inhibit NOS expression in a COX-2/PGE_2-independent fashion (21), indicate that drugs exhibiting selectivity towards both NOS and COX-2 may offer the potential for developing modified/new nonsteroidal antiinflammatory drugs with relatively little mechanism-based toxicity.

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