Aceclofenac Increases the Synthesis of Interleukin 1 Receptor Antagonist and Decreases the Production of Nitric Oxide in Human Articular Chondrocytes

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ABSTRACT. ***Objective.*** Interleukin 1 receptor antagonist (IL-1Ra) may play an important role in cartilage degradation by inhibiting IL-1 activity and therefore blocking IL-1 stimulation of prostaglandin E_2 (PGE_2) synthesis. Nitric oxide (NO) formation is increased during inflammation. High concentrations of NO exert negative effects on chondrocyte functions. We investigated the possible effects of 3 different nonsteroidal antiinflammatory drugs (NSAID; aceclofenac, piroxicam, aspirin) on IL-1Ra and NO production in human articular chondrocytes.

Methods. Normal and osteoarthritic (OA) cartilage samples were obtained from autopsy and prosthetic joint surgery, respectively. Chondrocytes were isolated and stimulated with 4 different stimuli: IL-1, tumor necrosis factor-α (TNF-α), lipopolysaccharide (LPS), and insulin-like growth factor (IGF). The 3 NSAID were added simultaneously to each different concentration of stimulus. IL-1Ra was measured in supernatant by ELISA; nitrites were quantified by the Griess reaction; PGE_2 level was measured by enzyme immunoassay.

Results. OA samples spontaneously produced higher levels of IL-1Ra than normal samples (130 ± 2.3 vs 30 ± 3.1 pg/ml). IL-1, TNF-α, and LPS produced dose dependent increases in synthesis of IL-1Ra. In their presence, IL-1Ra was detected in supernatant at 48 h, but its highest level was measured at 144 h. The most potent stimulus was IL-1, followed by TNF-α. Fetal bovine serum and IGF in turn did not modify the basal levels of IL-1Ra. In contrast to piroxicam and aspirin, aceclofenac 10 μg/ml and TNF-α 10 ng/ml increased almost 46 times the basal amount of IL-1Ra produced by OA chondrocytes. Additionally, aceclofenac and aspirin inhibited NO synthesis. Finally, the 3 NSAID reduced the levels of PGE_2 detected after stimulation with IL-1.

Conclusion. Proinflammatory stimuli induce IL-1Ra synthesis in human articular chondrocytes. Aceclofenac may modulate PGE_2 production by increasing IL-1Ra production and decreasing NO synthesis. Some NSAID exert diverse prostaglandin independent effects. (J Rheumatol 2001; 28:2692–9)

Key Indexing Terms:
HUMAN CHONDROCYTES   INTERLEUKIN 1 RECEPTOR ANTAGONIST
ACECLOFENAC   NITRIC OXIDE   PROSTAGLANDIN E_2 OSTEOARTHRITIS

The interleukin 1 (IL-1) family consists of 2 agonists, IL-1α and IL-1ß, and an antagonist, IL-1 receptor antagonist (IL-1Ra)[1]. IL-1 is a proinflammatory cytokine that plays an important role in articular inflammation and cartilage degradation[2]. IL-1 increases the synthesis of several inflammatory mediators such as nitric oxide (NO), prostaglandin E_2 (PGE_2), free oxygen radicals, metalloproteases, and other cytokines[2].

Various IL-1 inhibitory factors have been described. However, most have not been purified and/or their precise inhibitory mechanism remains to be established. One IL-1 inhibitor, IL-1Ra, specifically binds the IL-1 receptors of immune and inflammatory cells, but has no IL-1-like activity[3,4].

IL-1Ra is a 22 kDa protein that is produced by several cell types such as mature monocytes, macrophages, synoviocytes, and chondrocytes[3-6]. Production of the receptor antagonist may be mediated by cytokines present in inflammatory foci such as tumor necrosis factor (TNF) and IL-1[5,7,8]. IL-1Ra inhibits the biological action of IL-1[9]. The IL-1Ra blocks IL-1 stimulation of PGE_2 production in human synovial cells, and when IL-1Ra binds to the IL-1 receptor on chondrocytes, the induction of PGE_2 and tissue degrading enzymes by these cells is prevented.

Inhibition of the synthesis/activity of proinflammatory cytokine, such as IL-1, is an attractive option to decrease

inflammation and cartilage degradation in osteoarthritis (OA) and rheumatoid arthritis (RA)[10-12]. Several IL-1 inhibitors, such as tenidap, diacerhein, and IL-1Ra, have been reported to have beneficial effects on cartilage preservation and articular inflammation[13-15].

Cyclooxygenase inhibitors are used widely in the treatment of inflammatory arthropathies and represent the major pharmacological intervention in OA. Several effects of nonsteroidal antiinflammatory drugs (NSAID) have been described[12,16]: (1) inhibition of PGE_2 production; (2) inhibition of glycosyltransferase activity; (3) reduction of mitochondrial oxidative phosphorylation; (4) inhibition of IL-1 production and IL-1 receptor expression; (5) promotion of growth factor activity, including insulin-like growth factor I (IGF-I), which can be overcome by the inhibitory effect of IL-1; (6) increase of protein phosphorylation, which is dependent on the activation of cAMP dependent protein kinase; and (7) inhibition of metalloprotease synthesis and activity.

Aceclofenac is an NSAID that inhibits PGE_2 synthesis[17]. Further, aceclofenac reduces the production of IL-1 by synoviocytes[18]. Recently it has been reported that aceclofenac in the presence of IGF or/and serum may partially reverse the IL-1 inhibitory effect on glycosaminoglycan synthesis by cartilage tissue[19]. Some of these findings suggest that part of the inflammatory effects of aceclofenac are mediated by inhibiting IL-1 activity. To evaluate this mechanism, we studied the effect of aceclofenac on IL-1Ra synthesis by human chondrocytes.

MATERIALS AND METHODS

Tissue source. Normal adult human cartilage was obtained at autopsy from knees of 9 donors with no history of joint disease (age range 50–78 yrs). Human OA cartilage was obtained from 18 patients undergoing prosthetic joint surgery of femoral head (age range 55–81 yrs).

Chondrocyte isolation and culture. Cartilage slices were removed from the femoral heads and condyles, and washed in Dulbecco's modified Eagle's medium (DMEM). Tissues were then 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). Tissue was incubated on a shaker at 37ºC until the fragments were digested. Residual multicellular aggregates were removed by sedimentation (1× g). The cells were filtered through nylon mesh with pore diameter 25 μm and washed 3 times in RPMI 5% FBS, penicillin, and streptomycin before use. To ensure the chondrocyte phenotype, only high density primary passage cells were used.

Measurement of IL-1 receptor antagonist. Cells were planted into wells (250,000 cells in 24 well plate) with 0.5 ml of medium and incubated with the different stimuli: IL-1 (0.1, 1, and 5 ng/ml), TNF-α (0.1, 1, and 5 ng/ml), LPS (1, 10, and 100 μg/ml), and IGF (1, 10, and 100 ng/ml) for 24, 48, 72, and 144 h. NSAID (aceclofenac, aspirin, and piroxicam) at different concentrations were administrated simultaneously to each stimulus. Concentrations of NSAID chosen for these experiments were based on reported results[20]. Ethanol was used as a carrier for the NSAID, except aceclofenac, which was resuspended in distilled water, and carrier concentrations were maintained (< 0.01% v/v) in the cultures. IL-1Ra was measured in supernatant by ELISA (R&D Systems). The sensitivity of the assay was 14 pg/ml.

Quantification of nitrites. Chondrocytes were plated at 50,000 cells per well in 96 well plates and cultured for 48 h, and culture supernatants were collected for nitrite measurements. NO formation was detected by NO_2^- accumulation in the culture supernatants by the Griess reaction using sodium nitrite as standard. Briefly, 50 μl of culture supernatant were incubated with 50 μl 1% sulfanilamide, 0.1% N-1-naphthylethylenediamide dihydrochloride in 2.5% H_3PO_4 at room temperature for 5 min. Optical density was measured at 570 nm.

Measurement of PGE_2 levels. The conditioned medium from chondrocytes (cultured at 75,000 cells/well in 96 well plates in 0.1 ml medium) was collected after 24 h and stored at –80ºC. PGE_2 content was measured by enzyme immunoassay (EIA; Amersham Pharmacia Biotech) according to the manufacturer's instructions. The sensitivity of the assay was 40 pg/ml.

Data analysis. Results are presented as the mean of 6 individual experiments performed in duplicate wells. Each experiment was carried out separately with cells from the same donor. Results are expressed as mean ± SEM. Statistical analysis was performed with the unpaired 2 tailed Student t test.

RESULTS

Production of IL-1Ra by human articular chondrocytes. Human chondrocytes can synthesize IL-1Ra; however, the amount produced depends on the conditions to which they are subjected and the types of cells. Chondrocytes from human cartilage of subjects without joint disease spontaneously release very small amounts of IL-1Ra (30 ± 3.1 pg/ml) into the supernatant. In contrast, chondrocytes from OA cartilage synthesis yield levels 4 times higher (130 ± 2.3 pg/ml) than cells from normal cartilage (Table 1). The differences in the levels of production of IL-1Ra between normal and OA cells were even greater after stimulation with IL-1 (Table 1).

Modulation of IL-1Ra synthesis by several stimuli. OA is the most prevalent rheumatic disease and OA chondrocytes produce higher levels of IL-1Ra than normal cells. For these reasons we decided to analyze the effect of different stimuli on the production of IL-1Ra by human OA chondrocytes. We chose IL-1ß, TNF-α, and LPS according to results published with other types of cells. The results showed that the 3 proinflammatory stimuli we employed increased the release rate of IL-1Ra (Table 2). However, growth factors such as FBS and IGF-I had no stimulatory effect on the synthesis of IL-1Ra by human OA chondrocytes. The effects of proinflammatory stimuli were concentration dependent, and the optimal concentrations were IL-1ß at 5 ng/ml, TNF-α at 10 ng/ml, and LPS at 100 μg/ml (Figure 1). IL-1Ra was

Table 1. Production of IL-1Ra by human articular chondrocytes. Results are shown as the mean of 6 individual experiments and expressed as mean ± SEM.

Cell Donors	IL-1Ra, pg/ ml	
	Basal Level (Culture media)	Stimulated Level IL-1, 5 ng/ml
Normal	30 ± 3.1	230 ± 31.4
OA	130 ± 2.3	1,869 ± 38.1

Stimulus	IL-1Ra, pg/ml
Media	130 ± 2.3
FBS 5%	133 ± 1.9
FBS 10%	129 ± 2.8
IGF 10 ng/ ml	117 ± 4.3
IL-1, 5 ng/ml	1,869 ± 38.1
TNF, 10 ng/ ml	1,700 ± 43.4
LPS, 100 μg/ml	1,600 ± 40.7

FBS: fetal bovine serum; IGF: insulin-like growth factor; TNF-α: tumor necrosis factor; LPS: lipopolysaccharide.

detected in supernatant at 48 h, but it was observed that the optimal time to determine the accumulated level of IL-1Ra was at 144 h (Figure 2).

Effect of NSAID on IL-1Ra synthesis by chondrocytes. Apart from cyclooxygenase inhibition, other mechanisms have been attributed to some NSAID to explain their antiinflammatory properties. For this reason we studied modulation of the synthesis of IL-1Ra by NSAID, specifically aceclofenac, aspirin, and piroxicam. None of them was able to significantly modify basal levels of IL-1Ra produced by OA cells (Figure 3). However, combination of TNF-α with aceclofenac caused a 46-fold increase in the basal production of IL-1Ra. By contrast, combination of TNF with aspirin (10, 30, and 300 μg/ml) and piroxicam (1, 10, 100 μg/ml) did not significantly modify basal levels of IL-1Ra. The effect of aceclofenac was concentration dependent and the results
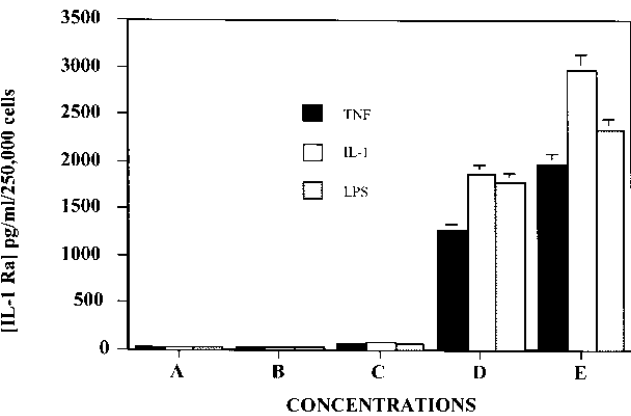

Figure 1. Dose response curve of the IL-1Ra synthesis by human articular chondrocytes. Chondrocytes from OA donors were stimulated under different conditions (250,000 cells/24 well plate). Concentrations of IL-1Ra were measured in supernatant after 144 h. A. Basal production of IL-1Ra. B. Levels of IL-1 Ra after stimulation with TNF-α 0.1 ng/ml, IL-1 0.1 ng/ml, and LPS 1 μg/ml. C. Levels of IL-1Ra after stimulation with TNF-α 1 ng/ml, IL-1 1 ng/ml, and LPS 10 μg/ml. D. Levels of IL-1Ra after stimulation with TNF-α 5 ng/ml, IL-1 5 ng/ml, and LPS 100 μg/ml. E. Levels of IL-1Ra after stimulation with TNF-α 10 ng/ml, IL-1 10 ng/ml, and LPS 500 μg/ml. Results are presented as the mean of 6 individual experiments and expressed as mean ± SEM.
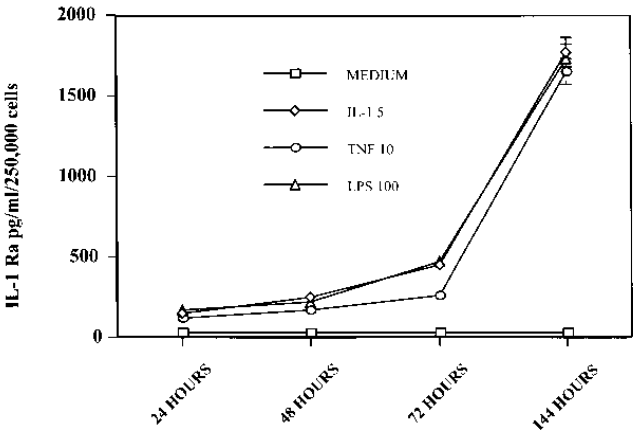

Figure 2. Time course of IL-1Ra synthesis by human articular chondrocytes. OA chondrocytes were cultured (250,000 cells/24 well plate) with 0.5 ml of medium alone or stimulated under different conditions: IL-1 5 ng/ml, TNF-α 10 ng/ml, or LPS 100 μg/ml. Concentrations of IL-1Ra were measured in supernatant after 24, 48, 72, and 144 h. Results are presented as the mean of 6 individual experiments and expressed as mean ± SEM.

showed that concentrations ≥ 5 μg/ml increased the amount of protein produced by OA chondrocytes (Figure 4).

Effect of aceclofenac on NO and PGE$_2$ synthesis in human chondrocytes. NO has been suggested as a factor that promotes cartilage catabolism in OA and proinflammatory responses in the joint. OA chondrocytes produce a larger amount of NO under proinflammatory cytokine stimulated conditions, such as with IL-1ß or TNF-α. Some NSAID are able to reduce the production of NO induced by IL-1ß on human chondrocytes. Aspirin, as reported, also reduces the synthesis of NO. However, piroxicam did not reduce the synthesis of NO on OA stimulated chondrocytes (Figure 5). Aceclofenac at concentrations of 5 and 10 μg/ml inhibited the NO production induced by IL-1ß by 40% and 70%, respectively, in human OA chondrocytes (Figure 6).

PGE$_2$ is an inflammatory mediator, and NSAID have the capacity to inhibit its synthesis. In additional experiments we analyzed the effect of aceclofenac, piroxicam, and aspirin on PGE$_2$ synthesis by human OA chondrocytes stimulated with IL-1ß. Cells were stimulated with IL-1ß in the presence and absence of NSAID, and the levels of PGE$_2$ and NO that accumulated in the same medium were examined. As expected, aceclofenac (Table 3), piroxicam, and aspirin (data not shown) inhibited the PGE$_2$ production on IL-1ß stimulated chondrocytes.

DISCUSSION

Although NSAID clearly inhibit the synthesis and release of prostaglandins, these actions are not sufficient to explain all their antiinflammatory effects[12,21]. We investigated the action of 3 NSAID, aspirin, aceclofenac, and piroxicam, on *in vitro* cytokine stimulated human chondrocytes producing IL-1Ra, NO, and PGE$_2$.

Table 3. Effects of aceclofenac on synthesis of inflammatory mediators. Results are shown as the mean of 6 individual experiments as mean ± SEM.

Stimulus	NO Level, nmol nitrite	PGE_2 Level, pg/ml
Control	0.94 ± 0.4	61.21 ± 6.3
IL-1, 5 ng/ ml	19.8 ± 2.3	603.86 ± 43.7
IL-1+ Ace, 1 μg/ml	19.4 ± 3.1	222.28 ± 31.9
IL-1+ Ace, 5 μg/ml	10.91 ± 2.9	114.85 ± 27.5
IL-1+ Ace, 10 μg/ml	5.97 ± 1.7*	81.88 ± 7.4*

Ace: aceclofenac. * $p < 0.01$.

The action of IL-1 is attenuated by at least 2 endogenous antagonists — IL-1Ra, a naturally occurring IL-1 inhibitor that binds to IL-1R without inducing biological responses, and the nonsignaling type II IL-1 receptor (IL-1RII), which is devoid of a cytoplasmic domain and serves as a decoy receptor[3,4,22,23]. Several studies have described the modulation of both proteins (IL-1Ra and IL-1RII) in rheumatic diseases and joint tissues[7,23-27]. Functional genomic analyses showed that soluble IL-1RII significantly inhibited IL-1ß induced NO and PGE_2 production in chondrocytes and synovial cells[23]. The studies of Dinarello and co-workers show that sIL-1RII plasma levels correlated negatively with indices of joint destruction. The levels of sIL-1RII were higher in patients with nondestructive arthritis than patients with destructive arthritis. In contrast, IL-1Ra plasma levels correlated positively with all indices of disease activity and joint destruction. For this reason IL-1Ra may be considered a natural antiinflammatory acute phase protein[24].

IL-1Ra levels are increased in > 80% of RA synovial fluid (SF) samples[28]. SF polymorphonuclear cells, chondrocytes, and synovial cells produce IL-1Ra, possibly
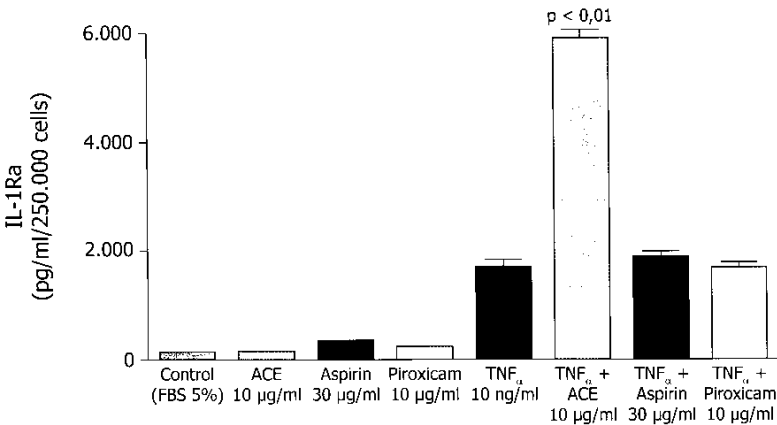

Figure 3. Effect of NSAID on synthesis of IL-1Ra induced by TNF. OA cells were incubated (250,000 cells/24 well plate) with TNF-α 10 ng/ml for 144 h. NSAID [aceclofenac (ACE), aspirin, piroxicam] were administered at different concentrations simultaneously with TNF-α. IL-1Ra was measured in supernatant by ELISA. Results are presented as the mean of 6 individual experiments and expressed as mean ± SEM. FBS: fetal bovine serum control.
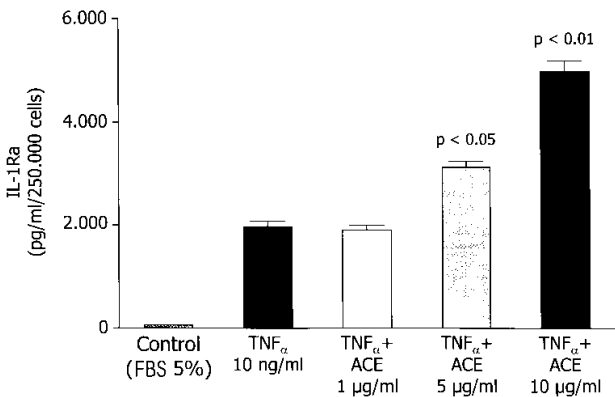

Figure 4. IL-1Ra production: dose response curve with aceclofenac (ACE). OA cells were incubated (250,000 cells/24 well plate) with TNF-α 10 ng/ml for 144 h. Aceclofenac at different concentrations was administered simultaneously with TNF-α. IL-1Ra was measured in supernatant by ELISA. Results are presented as the mean of 6 individual experiments and expressed as mean ± SEM. FBS: fetal bovine serum control.
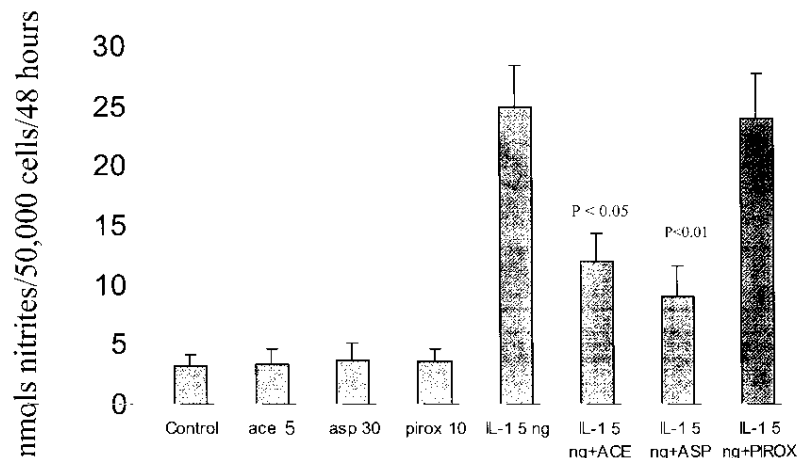
Figure 5. Effect of NSAID on NO production. OA cells were incubated (50,000 cells/96 well plate) with IL-1 5 ng/ml for 48 h. NSAID at different concentrations [aceclofenac (ACE) 5 μg/ml, aspirin (ASP) 30 μg/ml, piroxicam (PIROX) 10 μg/ml] was administered simultaneously with IL-1. NO was measured in supernatant by Griess reaction. Results are presented as the mean of 6 individual experiments and expressed as mean ± SEM.
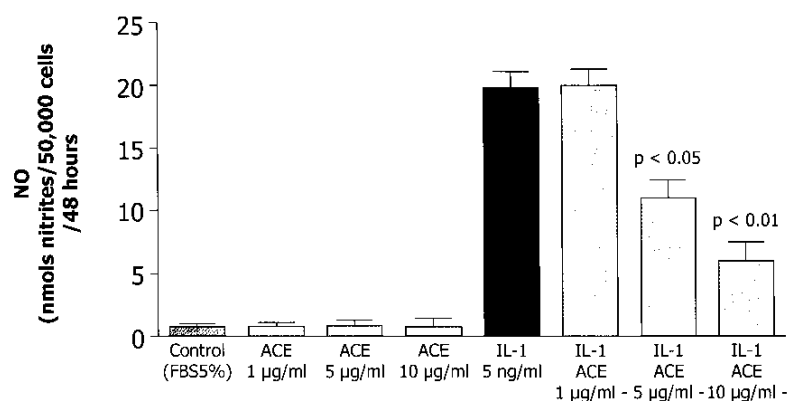

Figure 6. NO production: dose response curve with aceclofenac (ACE). OA cells were incubated (50,000 cells/96 well plate) with IL-1 5 ng/ml for 48 h. Aceclofenac at different concentrations was administered simultaneously with IL-1. NO was measured in supernatant by Griess reaction. Results are presented as the mean of 6 individual experiments and expressed as mean ± SEM. FBS: fetal bovine serum control.

contributing to the levels of IL-1Ra present within the SF[7,26-29]. Human normal and OA chondrocytes are able to produce IL-1Ra, and IL-1 stimulates IL-1Ra synthesis in both OA and normal chondrocytes. Pelletier, *et al* have reported that spontaneous production of IL-1Ra is increased in OA cartilage[26]. To our knowledge there is no study comparing the synthesis of IL-1Ra on human normal and OA chondrocytes. We have found that the pathologic condition of the cartilage has an influence on the synthesis of IL-1Ra. The results showed that OA cells spontaneously synthesized higher amounts of IL-1Ra than normal cells, and that proinflammatory stimulus increased the production of IL-1Ra on both normal and OA chondrocytes. The reason OA chondrocytes produce higher levels of IL-1Ra *in vitro* than normal chondrocytes may be related to the presence, *in situ*,

of cytokines such as IL-1 that may induce the expression of IL-1Ra production. Moreover, OA chondrocytes appear to be hyperresponsive to IL-1 stimulation, and this seems to be related to an increased level of IL-1 receptor in OA chondrocytes. A similar phenomenon has been reported with respect to metalloproteases and NO synthesis, relating this to an increased level of IL-1 receptor in OA chondrocytes[25]. Plasma IL-1Ra levels are significantly higher in patients with chronic polyarthritis than in healthy controls, suggesting that increased levels of IL-1Ra may reflect increased production and activity of IL-1[24]. Further, cytokines such as IL-4 or IL-10 may also contribute to the balance between the production of IL-1 and that of IL-1Ra[1].

We showed also that growth factors such as FBS and IGF-I are not able to modify either spontaneous or proin-

flammatory production of IL-1Ra; and that chondrocyte synthesis of IL-1Ra responded to IL-1ß, TNF-α, and LPS stimulation in a concentration dependent manner.

IL-1 plays an important role in inflammation, cartilage degradation, and probably also in the physiopathology of OA and RA[2,11,12]. A relative deficit of IL-1Ra has been shown in both OA and RA. This deficit may be a possible explanation for an increased level of IL-1 activity, leading to enhancement of cartilage matrix degradation. The exact reason(s) for the discordant expression and synthesis of IL-1 and IL-1Ra under these pathological conditions remains unknown. Some authors think that in OA and RA pathology the production of IL-1Ra is increased[24,26,30]. However, its synthesis is insufficient in relation to the excessive amounts of IL-1 produced locally, and this imbalance is responsible for the predominance of the catabolic process in OA and RA. Other authors did not detect levels of IL-1Ra or IL-1RII in human OA chondrocytes[23]. They propose that cartilage degradation in OA occurs because OA chondrocytes lack a natural defence mechanism against endogenous IL-1.

Either reducing IL-1 production or blocking its actions is an appropriate strategy for treating acute and chronic rheumatic diseases[1,10,11]. Aceclofenac, but not aspirin or piroxicam, increases IL-1Ra production by TNF-α stimulated OA chondrocytes. Pelletier, et al reported that aceclofenac inhibits IL-1 synthesis on LPS stimulated fibroblast synovial cells[18]. Overall, aceclofenac can be of benefit by delaying cartilage degradation and blocking some inflammatory activities of IL-1. Other NSAID like mofezolac induced IL-1Ra synthesis in cultured human peripheral blood mononuclear cells. The mofezolac stimulated mRNA expression of IL-1Ra[31].

Exogenously added IL-1Ra is quite effective in blocking IL-1ß mediated effects on chondrocytes and in inhibiting PGE_2 release by chondrocytes. In vitro experiments revealed that an excess of 10 to 100 times the amount of IL-1Ra is necessary to inhibit 50% of IL-1 activity[1,32,33]. Stimulation of cells with TNF-α and aceclofenac increases almost 46 times the basal levels of IL-1Ra.

On the other hand, excessive concentrations of NO exert profound effects on chondrocyte functions, including downregulation of collagen synthesis, chondrocyte proliferation[34], inhibition of actin polymerization[35], downregulation of IL-1Ra expression[26], activation of metalloproteases[36], and induction of apoptosis[37]. These manifestations induced by NO may be a possible mechanism of cartilage degradation in arthritis. Recently, effects of current antiinflammatory drugs on NO production have emerged as an additional property of these agents[38]. The capacity to inhibit NO synthetase is not common to all NSAID. The consistent finding in the literature is that salicylates, but typically not indomethacin, inhibit NO production in a variety of cell types, including chondrocytes and macrophages, most likely through effects on translation[38]. Our observation indicates that aceclofenac, as well as aspirin, is also an effective inhibitor of NO production, and it suggests that this is not a property of salicylates alone and may be shared by other classes of antiinflammatory agents.

Prostaglandin levels are elevated in SF and tissues of patients with RA. Prostaglandins mediate the characteristic vasodilation and erythema observed in acute inflammation. PGE_2 stimulates production of matrix metalloproteinases by synoviocytes that leads to destruction of cartilage. NSAID classically act by inhibiting the key enzyme of prostaglandin synthesis, which we now call cyclooxygenase or COX. All NSAID tested in this study inhibited prostaglandin production.

An important property of NO in inflammation is its ability to activate COX, resulting in substantial production of proinflammatory prostaglandins[34,39]. Regarding cartilage tissue and chondrocytes, there are contradictory reports on the effect of NO on PGE_2 production. We have shown that NO strongly increases PGE_2 production by human chondrocytes[34]. Other authors, however, suggest that NO suppresses PGE_2 synthesis[40]. Although the connection between NO and PGE_2 is confusing, these effects are not due to the shared capacity to inhibit COX-2 dependent prostaglandin production in these cells, since neither indomethacin nor piroxicam (at concentrations sufficient to inhibit PGE_2 production) inhibited NO release. Further, NSAID (such as aspirin and aceclofenac) that inhibit PGE_2 production do not increase the synthesis of NO. Aspirin blocks NO expression at the translational level, and probably also directly at the enzyme level by acetylating the enzyme to render it inactive[38]. We do not yet know the pathway of aceclofenac to block NO synthesis in chondrocytes.

NO markedly reduced the synthesis of IL-1Ra. The effect of NO downregulates the production of IL-1Ra at the gene expression level[26]. The exact mechanism responsible for the effect of NO on IL-1Ra expression and synthesis is unknown. Aceclofenac could increase IL-1Ra levels through its negative effect on NO synthesis. However, this is unlikely, because other NSAID such as aspirin that inhibit NO synthesis have not shown any effect on IL-1Ra production. The possibility that inhibition of NO and PGE_2 production by aceclofenac is due to stimulation of expression of IL-1Ra cannot be ruled out by our experiments.

This study has some limitations. We used cells and not cartilage tissue. Perhaps a study in the natural environment of chondrocytes would demonstrate the potency of NSAID on cartilage, and also show the ability of NSAID to penetrate the cartilage. This approach is being explored in our laboratory.

In summary, we showed that chondrocytes responded in a concentration dependent manner on IL-1Ra synthesis upon stimulation with IL-1ß, TNF-α, and LPS. We also demonstrated that aceclofenac, which is known to inhibit the synthesis of IL-1ß, also inhibits the production of other

inflammatory mediators such as NO and PGE_2, and it also increases IL-1Ra production. This could be an additional mechanism by which aceclofenac produces its antiinflammatory effect and protects against the cartilage degradation process in arthritis.

ACKNOWLEDGMENT

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