Inflammation in Osteoarthritis

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In 1979, Palmoski and Brandt[1] raised the possibility that some nonsteroidal antiinflammatory drugs (NSAID) had deleterious effects on articular cartilage metabolism. They noted that salicylate affected the metabolism of normal canine articular cartilage *in vitro*, and demonstrated that the greatly augmented rate of proteoglycan synthesis in OA cartilage in the canine cruciate-deficiency model of OA was inhibited by salicylate. The Indiana group subsequently also showed that other NSAID — but not all — markedly reduced the augmented levels of proteoglycan synthesis in canine OA cartilage. Further, *in vivo*, administration of salicylate accelerated the development of structural damage in the OA joint in the canine cruciate-deficiency model and resulted in more severe pathology than that seen in the OA knees of dogs not treated with the drug. Brandt and his colleagues showed that one of the mechanisms underlying this effect was the inhibition by salicylate of glucuronyltransferase, an enzyme required for the conversion of glucose to glucuronic acid, which is essential for elongation of the chondroitin sulfate chains of cartilage proteoglycans[2]. Further, this effect was shown to be prostaglandin (PG)-independent. In my view, these studies represent the birth of this field.

Figure 1 depicts the results of a study by Dingle[3], examining the effects of a variety of NSAID on explants of OA cartilage. The results led to the conclusion that NSAID fell into 3 groups: those that enhanced proteoglycan synthesis, those whose effect was neutral, and those, such as aspirin and the propionic acid derivatives, that inhibited proteoglycan synthesis. Although with such studies the caveat always exists that the concentration of drug employed *in vitro* may not have been comparable to the tissue concentrations present *in vivo*, the notion that NSAID differ with respect to their effects on cartilage, however, is valid. Relatively few data exist in this regard with respect to selective cyclooxygenase-2 (COX-2) inhibitors. However, Masterbergen, *et al*[4] found recently that the decreased proteoglycan concentration of OA cartilage and increased release of proteoglycan from the tissue *in vitro* were normalized over 7 days in the presence of celecoxib, while the level of proteoglycan synthesis was unaffected, suggesting that this drug may not have an adverse effect on the metabolism of OA cartilage.

Arthroscopic studies have provided some intriguing observations on the relationship of synovitis to cartilage damage in OA. Dougados, *et al*[5] reported that arthroscopy detected clinically unrecognized synovitis in up to one-third of patients with radiographic changes of OA and that the sites of synovial inflammation abutted on the cartilage lesions (Figure 2). Further, longitudinal arthroscopic studies supported that synovitis was associated with the progression of cartilage damage in OA. Young, *et al*[6] demonstrated the expression of chemokines, collagenases, and stromelysin in areas of synovitis in OA joints. It is not surprising, therefore, that synovitis may result in progressive destruction of adjacent articular cartilage.

Do NSAID affect synovial collagenase production? In the light of cancer research studies that have indicated that PG activate matrix metalloproteinases (MMP) in the tissues, we addressed this question. When we examined the release of collagenases (MMP-1, MMP-9, MMP-13) from synovial fibroblasts exposed to interleukin 1 (IL-1) and tumor necrosis factor-α (TNF-α) (Figure 3), we found that high concentrations of aspirin inhibited the release of MMP into the culture medium, perhaps related to the ability of this drug to inhibit MAP-kinase[7]. In contrast, addition of indomethacin or ibuprofen doubled the amount of MMP-1 release by the cells. Immunoblots indicated that addition of PGE_1 and PGE_2, but not $PGF_{2\alpha}$, to the medium prevented the upregulation of MMP-1 induced by the cytokines. It is clear that different eicosanoid products exhibit different biological effects; we cannot lump together the actions of different PG.

As shown in Figure 4, the COX-2 selective inhibitor SC299 enhanced cytokine-stimulated release of MMP-1, which indicated that COX-2-derived PG exert an inhibitory effect on MMP release. Consistent with that conclusion, addition of the end-product, PGE_1, reversed the enhancement of MMP-1 production by SC299.

We found that low concentrations (< 1 mM) of aspirin, although sufficient to inhibit PG synthesis, did not inhibit activity of the MAP-kinase, extracellular signal-related kinase (ERK), but did enhance MMP-1 release. At higher (ERK-inhibiting) concentrations, MMP-1 release was also inhibited. In contrast, sodium salicylate, which did not inhibit PG production at low concentrations, did not increase MMP-1 production (Figure 5). However, at high concentrations, sodium salicylate, like aspirin, inhibited both ERK and MMP release from cytokine-stimulated synoviocytes. The effect of salicylates on the release of MMP-9 was similar to that for MMP-1; however, release of MMP-13 was unaffected by addition to the culture medium of salicylates or other NSAID, or of PGE.

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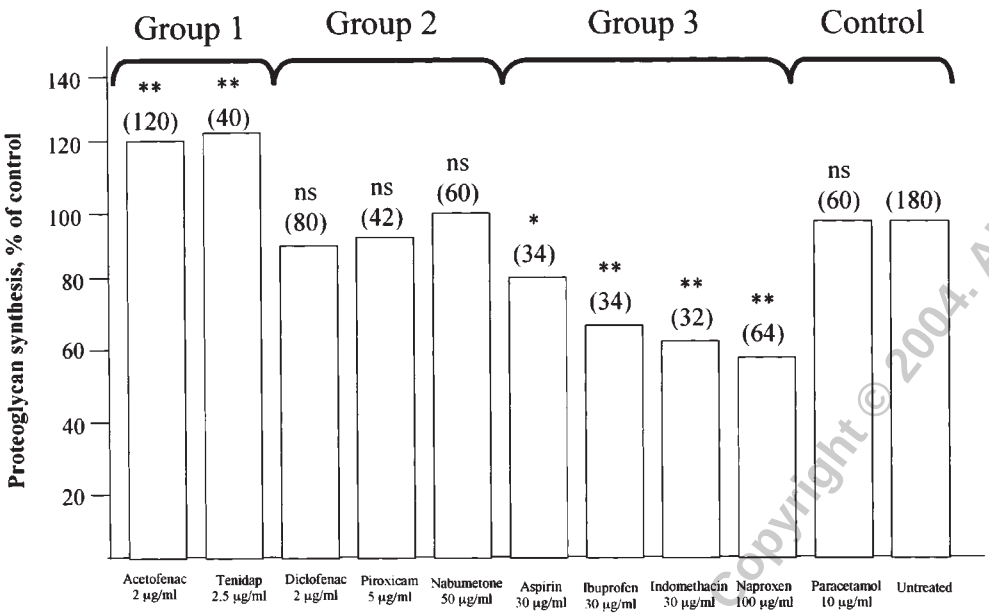

Figure 1. Effects of various NSAID on proteoglycan synthesis by articular cartilage from patients with OA. Group 1: drugs that stimulate proteoglycan synthesis; Group 2: NSAID that had no significant effect on proteoglycan synthesis; Group 3: drugs that inhibited proteoglycan synthesis; Group 4: controls paracetamol and no drug. The values in parentheses represent cartilage samples analyzed. *p < 0.05; **p < 0.01; ns: not significant. With permission, from Dingle. Arthritis Rheum 1979;22:746-54.

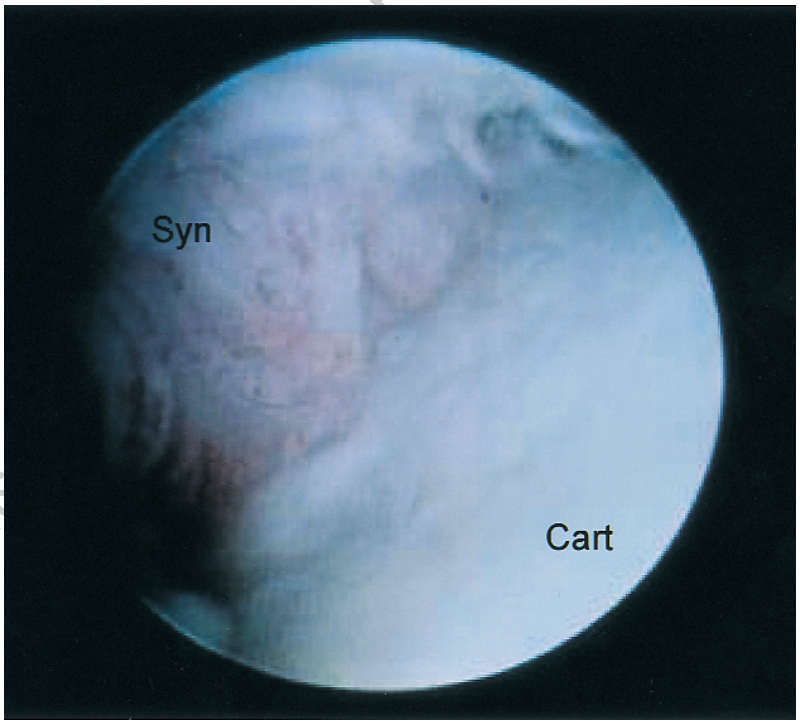

Figure 2. Arthroscopic view of the knee of a patient with OA showing the proximity of synovitis to a site of chondropathy. Syn: inflammatory synovial membrane. Cart: Articular cartilage of the trochlear groove. Figure kindly provided by Drs. X. Ayral and M. Dougados. From Ayral, *et al*. J Rheumatol 1999;26:1140-7.

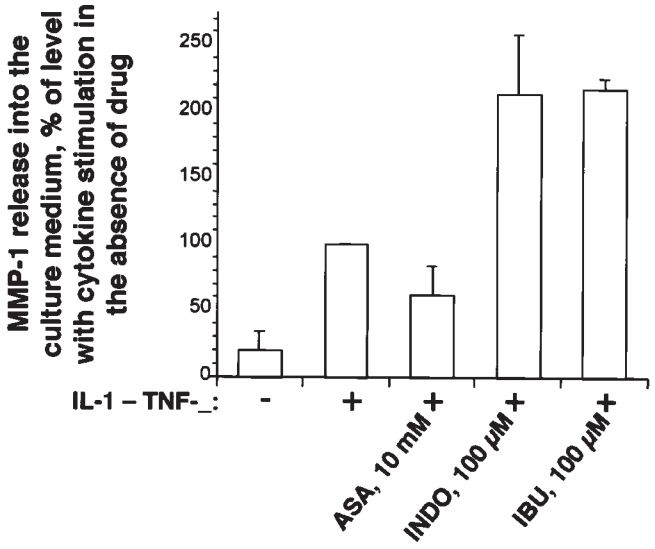

Figure 3. Aspirin (ASA) inhibits, but indomethacin (INDO) and ibuprofen (IBU) promote the release of MMP-1 from synoviocytes stimulated with IL-1/TNF-α. With permission, from Pillinger, *et al*. J Immunol 2003;171:6080-9.

Thus, as illustrated in Figure 6, the data indicate that endogenous PG exert an inhibitory effect on ERK activation, which causes suppression of MMP-1 production. At lower (< 1 mM) concentrations, NSAID, coxibs, and aspirin, by virtue of their capacity to block PGE production, permit enhanced ERK activity and hence greater MMP-1 release. At higher concentrations, however, both aspirin and sodium salicylate inhibit ERK activation. This PG-independent effect of salicylates results in an inhibition of cytokine-induced MMP-1 release. Several years ago, Brandt and his colleagues[2] pointed out that sodium salicylate inhibited proteoglycan synthesis in articular cartilage and that, as noted above, even at high concentrations this appeared to be a PG-independent effect.

The above studies illustrate an effect on collagenase production by an NSAID. However, the clinical importance of these observations is unknown. In any event, it appears that PGE_1 tonically suppresses collagenase release, i.e., it is a "good prostaglandin." When we administer an NSAID — regardless of what we achieve with respect to the normalization of clinical signs of inflammation — we inhibit PG. The result, however, may be enhanced release of MMP in joint tissues and elsewhere.

Many similar studies have been published in which the concentrations of some products have been shown to be increased and those of others, decreased. The net effect is unclear. The only way we will be able to determine which of these effects are important and which are not will be through validation of the basic findings in randomized clinical trials. Unfortunately, the published clinical trial literature in this area is not helpful. Why are we unable to draw conclusions from the data generated by these clinical trials?

In a 2-year clinical trial in which 178 patients were randomized to treatment with either naproxen or acetaminophen, only 62 completed the trial[8]. The remainder discontinued treatment because of lack of efficacy or adverse events. As illustrated by this study, it is difficult to maintain the majority of OA subjects taking an NSAID or acetaminophen in a blinded clinical trial for a protracted period (e.g., > 3 months) without a large proportion discontinuing treatment because of lack of efficacy or adverse effects. Because joint space narrowing (a surrogate for cartilage loss) develops very slowly in OA, if it is the outcome measure of a clinical trial, the problem of patient retention over the course of 1–3 years is formidable.

In the above study, about two-thirds of those who

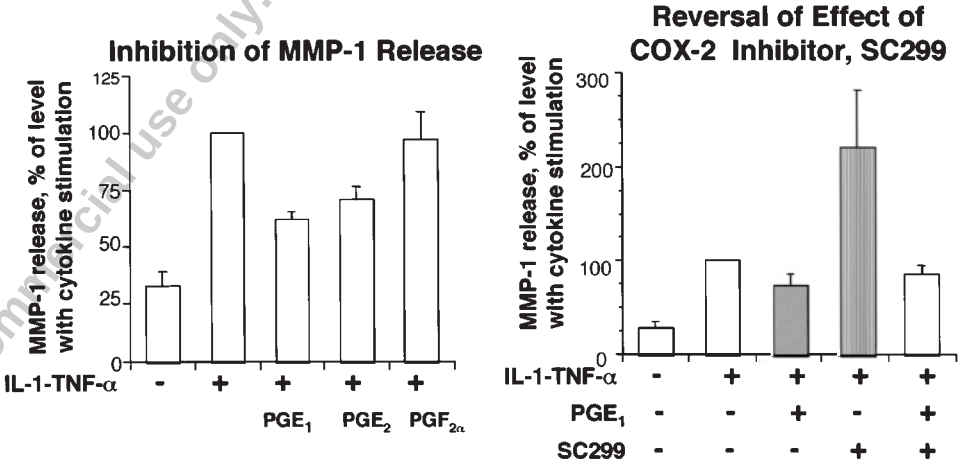

Figure 4. E series prostaglandins, but not $PGF_{2\alpha}$, inhibited release of MMP-1 from cytokine-stimulated synoviocytes (left panel), and reversed the increase in MMP-1 release produced by the selective COX-2 inhibitor SC 299 (right panel). With permission, from Pillinger, *et al*. J Immunol 2003;171:6080-9.

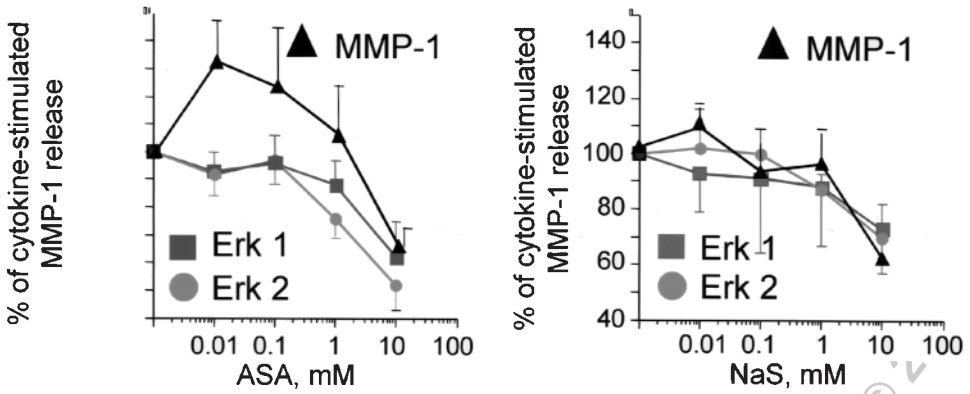

Figure 5. Low concentrations of aspirin (ASA), but not of sodium salicylate (NaS), enhanced the release of MMP-1 from synoviocytes stimulated with IL-1/TNF-α, while higher concentrations of aspirin inhibited MMP-1 release. Erk-1 and Erk-2: mitogen-activated protein kinases (MAPK). With permission, from Pillinger, *et al*. J Immunol 2003;171:6080-9.

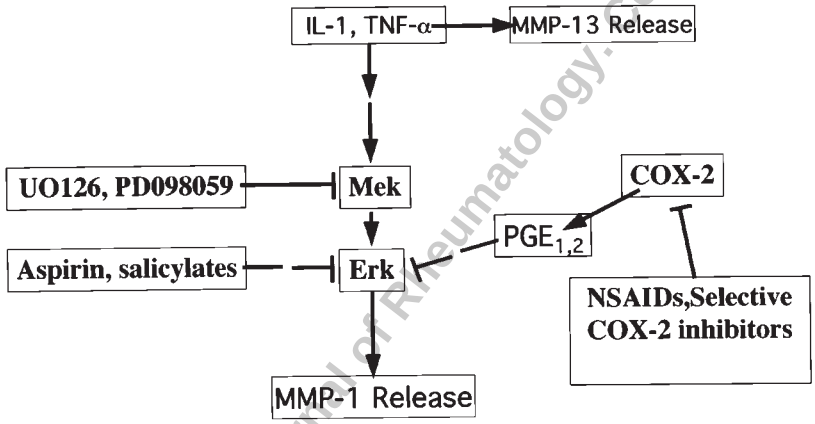

Figure 6. Model for the regulation of MMP-1 expression by extracellular signal-related kinase (ERK), and the regulation of ERK by salicylates and prostaglandins. The data indicate that production of MMP-1, -3, and -9 in response to IL-1ß and/or TNF-α depends upon ERK activation, and that PGE production by COX-2 downregulates ERK-mediated production of MMP-1. Agents that inhibit COX (including < 1 mM ASA) therefore enhance ERK activation and secondarily enhance production of MMP-1, whereas agents that inhibit the ERK pathway (including > 1 mM ASA and sodium salicylate) inhibit production of MMP-1 (and MMP-3 and -9). These effects may be exerted at the level of Mek, a kinase that regulates ERK activation and is the site of action of "ERK inhibitors," e.g., U0126, PD098059. MMP-13 is also activated by IL-1ß/TNF-α in an ERK-independent manner. Because IL-1ß/TNF-α not only activates ERK but also stimulates PGE production via COX-2 upregulation, the possibility of a regulatory loop is suggested by these data.

completed the trial reported that their symptoms were worse or unchanged, regardless of whether they were treated with acetaminophen or naproxen. The authors found essentially no difference between these 2 drugs and — offering a caveat about the small number of subjects available for analysis at the end of the study — concluded, on the basis of a comparison of knee radiographs obtained at baseline and 2 years later, that there was no difference between the 2 treatment groups with respect to the progression of structural joint damage.

In 1993, Dieppe, *et al*[9] undertook a 2-year placebo-controlled trial of an NSAID in patients with knee OA, in which 89 patients were randomly allocated to receive slow-release diclofenac 100 mg/day or matching placebo. Thirty-eight patients withdrew or dropped out of the study prematurely, with most of the dropouts occurring within the first 6 months. No radiographic change was apparent in most of the 51 patients who completed the trial. However, standard radiographic procedures were employed in that study. It has subsequently become clear that the knee radiographic protocol employed does not reproducibly standardize the radioanatomic positioning of the knee, so that

any conclusion with respect to radiographic progression, especially given the small number of subjects in this trial, must be regarded with great caution.

In 1995, Huskisson, *et al*[10] presented the results of another clinical trial, the LINK Study, in which 812 patients were randomized to treatment with indomethacin or placebo or to treatment with tiaprofenic acid or placebo. Three hundred and seventy-six patients (fewer than half) completed at least one year of treatment. The authors reported that 22% of the placebo group, but 47% of subjects in the indomethacin group, showed progression of radiographic changes of OA, while the incidence of radiographic progression with tiaprofenic acid was not significantly different from that with placebo (43% and 34%, respectively). The authors concluded that indomethacin accelerated structural damage in OA and terminated the study prematurely. Although this report, at first glance, would appear to confirm the concept of analgesic arthropathy, i.e., patients with OA given indomethacin exhibited accelerated progression of their OA, great caution is needed in interpretation of the results in view of the large dropout rate and the fact that, as in the study cited above, the radiology protocol did not provide the level of reproducibility in positioning of the knee that can now be achieved with protocols that have been developed subsequently.

In a study reported by Buckland-Wright, *et al*[11], 45 patients with knee OA were randomized to treatment with diclofenac or placebo for 18 months; 33 (17 of whom received NSAID and 16 placebo) completed the study. High definition macroradiography was performed at 6-month intervals, with the knee reproducibly positioned in the semi-flexed AP view. Evaluation of medial tibial compartment joint space width revealed no statistically significant differences between the 2 treatment groups.

Lane, *et al*[12] recently performed a trial in 60 patients with knee or hip OA who were randomized to NSAID treatment or acetaminophen and followed every 6 months for 3.5 years. The 2 treatment groups were indistinguishable with respect to deterioration in scores on the Health Assessment Questionnaire (HAQ) or changes in their knee radiographs.

Thus, following the sentinel observations made by Brandt and his colleagues more than 20 years ago, the question whether NSAID alter the progression of structural damage in the OA joint remains unanswered. Some NSAID can be shown reproducibly to adversely affect cartilage or synovial metabolism *in vitro* but, with the exception of the LINK study (see above), prospective clinical trials have not indicated a deleterious effect of NSAID on the progression of OA. It is not clear, however, whether the absence of evidence is, in fact, evidence of the absence of an effect. It is very difficult to perform longterm radiographic studies of patients receiving NSAID therapy. Until we have better outcome measures, we probably will not learn the answer to this question.

Dieppe suggests elsewhere in these proceedings that OA should be thought of as a mechanically-driven, but chemically-mediated, disease in which repair represents a dominant aspect of the pathogenetic process[13]. I would, therefore, like to consider chemically-mediated aspects of the inflammatory component of OA that are potential targets for intervention that might be addressed while we are trying to improve joint mechanics.

Goldring[14] has provided a useful schematic view of the processes involved, including the effects of tissue inhibitors of metalloproteinases, plasminogen activator inhibitor, and MMP (Figure 7). I will define the inflammatory aspect of OA in a manner somewhat different from the usual.

It seems clear that mechanical forces produce metabolic changes that result in cytokine production by the synovium. With the persistence of mechanical stress on joint tissues, inflammation develops. However, because the cartilage is not vascularized or innervated, this may not manifest itself as joint swelling or pain. Nonetheless, it results in the production of a host of mediators that we ordinarily associate within inflammatory arthritis and which act in a paracrine and autocrine fashion to endow the chondrocytes with a catabolic phenotype. In some patients, these mediators are produced by synovial cells, in others, by the chondrocytes, and in yet others, by both. PG are key mediators in this process. The superficial zone of articular cartilage from patients with either OA or rheumatoid arthritis shows positive immunostaining for IL-1, TNF, and inducible nitric oxide synthase (iNOS)[15]. Thus, mechanical stress leads to an increase in the levels of chemical mediators of inflammation in cartilage.

Fermor, *et al*[16] have shown that intermittent compression and, under some conditions, static compression may result in upregulation of iNOS, which is not detectable in unloaded chondrocytes. The amount of iNOS protein produced in cartilage cells under compression is as great as that seen in cultured macrophages exposed to interferon or endotoxin. Under certain conditions of compression, the induction of IL-1 and MMP in chondrocytes can also be demonstrated.

Several years ago we showed that, in contrast to normal cartilage, OA cartilage spontaneously produces significant quantities of PGE_2[17] and an array of cytokines (Figure 8). PGE_2 production by normal chondrocytes can be upregulated by addition of IL-1 and other cytokines. Conversely, the effects of the cytokines can be abolished.

Recent observations by Simmons, *et al*[18] suggest the presence of a COX-3 isoform (possibly a spliced variant of COX-1). The important point is that COX inhibitors may have a variety of targets upon which to act, and the activities of the COX isoforms may result in a variety of end-products that we tend to regard as cell-specific. Thus, we speak about PGE_2 in inflammation, about thromboxane in platelets, and about prostacyclin in the endothelial cells of blood vessel walls. The chondrocyte, however, has the capacity to produce virtually all of the various eicosanoids, including PGE_2, PGD_2, prostacyclin, thromboxane, and leukotriene B_4

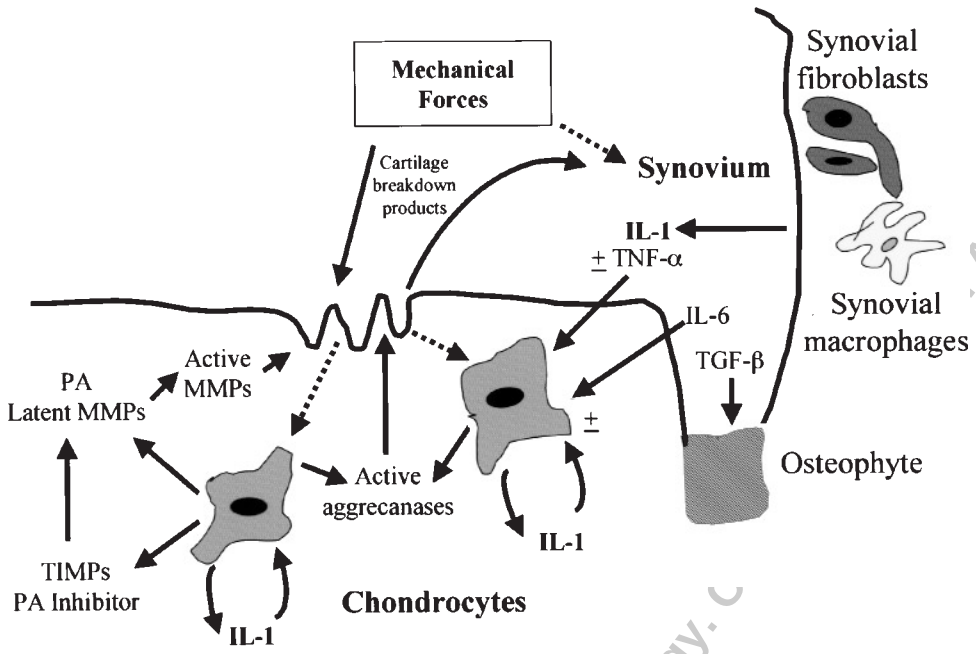

Figure 7. Chemically-mediated aspects of the inflammatory component of OA. PA: plasminogen activator; TIMP: tissue inhibitors of matrix metalloproteinases. With permission, from Goldring, *et al*. Arthritis Rheum 2000;43:1916-26.

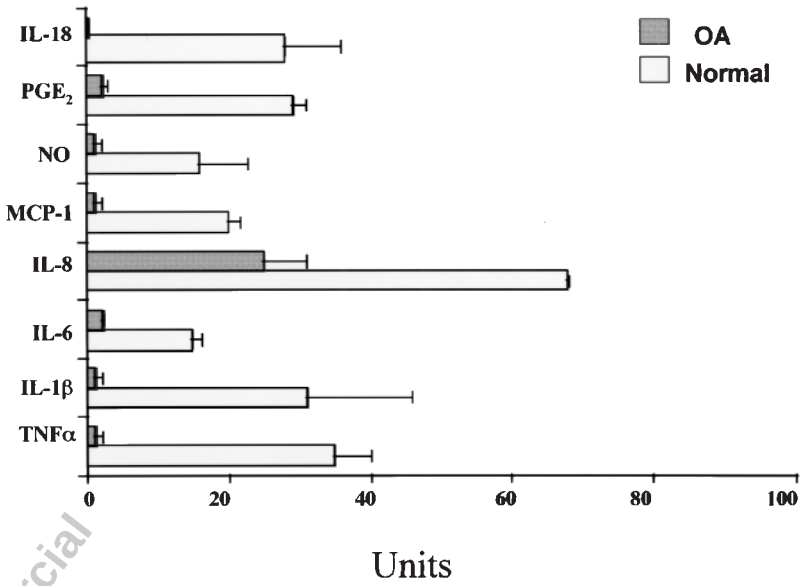

Figure 8. Spontaneous production of inflammatory mediators by normal and OA cartilage. With permission, from Attur, *et al*. Osteoarthritis Cartilage 2002;10:1-4. MCP-1: monocyte chemoattractant protein-1.

(LTB_4)[19]. This wide spectrum of end-products can be expected to have a variety of biologic activities — particularly PGD_2, which may be converted to PGJ_2, a recently described inhibitor of the effects of IL-1 in chondrocytes and synovial cells[20], and products of the lipoxygenase pathway, including LTB_4 and lipoxin.

We have shown that NSAID inhibit PGE_2 and PGD_2 production by OA cartilage. What was of particular interest in that experiment, however, was a concomitant quadrupling of the amount of LTB_4 produced when COX-2 was inhibited, due to shunting into the lipoxygenase pathway[19]. Not only selective COX-2 inhibitors, but also nonselective

NSAID, such as indomethacin, upregulate LTB_4. The effects of LTB_4 production on cartilage remain to be determined. However, as a result of shunting from one pathway into another, we may create an unexpected effect on cartilage metabolism. When we studied PG production by fibroblasts derived from COX-2 knockout mice, which produce only the COX-1 isoform, it was apparent that the pathway that consumed most of the arachidonic acid generated increased amounts of LTB_4. These observations mimic the effects we observed when we inhibited COX-2 with indomethacin or celecoxib.

To add to the complexity and to indicate why it is hard to determine the effects on cartilage of PG synthase inhibition it is worth noting that we do not yet fully understand where COX localizes within the cell. One might think it should be localized to the cell membrane, where its action can result in the production of heat, redness, swelling, and pain. In fact, however, 90% of COX-1 and COX-2 are localized around the nucleus of the chondrocyte and in the Golgi apparatus, indicating that, in addition to the secretion of PG, a primary effect of COX activation is the regulation of gene transcription.

Under controlled conditions, we have examined cytokine-related gene expression in cartilage explants in the presence of COX-2 inhibition. As expected, we demonstrated a decrease in PG production. COX-2 levels, however, are in a sense merely a convenient marker that we can use to measure the activity of an NSAID; addition of a COX-2 inhibitor affects many inflammatory genes — upregulating some and inhibiting others. For example, the level of gene transcripts for intercellular adhesion molecule-1 doubled in the presence of a COX-2 inhibitor. Similarly, chemokines, cytokines, proteases, protease inhibitors, factors that regulate apoptosis, and growth factors (e.g., osteopontin, fibroblast growth factor) were upregulated, while the activity of various basic housekeeping genes was unaffected.

Identification of specific actions of individual eicosanoid end-products could, theoretically, lead to more targeted intervention in the treatment of OA (e.g., to an LTB_4 inhibitor) that could provide a better understanding of the influence of NSAID on disease progression. Clearly, we need better clinical studies. It is impossible to predict from *in vitro* studies the longterm effects on cartilage of various eicosanoid inhibitors. Further, better clinical data may lead to the production of more specific inhibitors, which could minimize the adverse effects of therapy.

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