

Leukotriene and Prostaglandin Synthesis Pathways in Osteoarthritic Synovial Membranes: Regulating Factors for Interleukin 1 β Synthesis

PATRICK MARCOUILLER, JEAN-PIERRE PELLETIER, MÉLANIE GUÉVREMONT, JOHANNE MARTEL-PELLETIER, PIERRE RANGER, STEFAN LAUFER, and PASCAL REBOUL

ABSTRACT. *Objective.* To study the mechanisms responsible for the cross-talk between lipoxygenase (LOX) and cyclooxygenase (COX) pathways in human osteoarthritic (OA) synovial explants, and to confirm the arachidonic acid (AA) shunting phenomenon and its influence on interleukin 1 β (IL-1 β) synthesis. *Methods.* Synovial membrane explants were cultured in the absence or presence of different drugs that inhibit COX and/or LOX activities. Concentrations of prostaglandin E₂ (PGE₂), leukotriene B₄ (LTB₄), lipoxin A₄ (LXA₄), and IL-1 β were measured. *Results.* When membrane explants were incubated with naproxen (COX inhibitor) under unstimulated conditions, the production of LTB₄ was dose-dependently enhanced, reaching a 5-fold increase over the control. This shunt could be partially reversed by the addition of exogenous PGE₂. Under lipopolysaccharide (LPS) stimulation, both licofelone (COX/LOX inhibitor) at therapeutic concentrations and NDGA (LOX inhibitor) inhibited LTB₄ production, whereas naproxen did not amplify the LPS-induced LTB₄ production. Conversely, using NDGA, it was found that a shunt of AA from the LOX to the COX pathway did not occur. Under LPS conditions, both naproxen and licofelone inhibited LXA₄, inducing an increase in the LTB₄/LXA₄ ratio with naproxen treatment but not with licofelone. Under these conditions, naproxen treatment induced a higher level of IL-1 β production. *Conclusion.* We demonstrated in OA synovium that a shunt from AA to the LOX pathway occurred and that treatment with a nonselective COX inhibitor could increase the production of LTB₄ and secondarily the synthesis of IL-1 β . Therefore treatment with licofelone, which can act on both COX and LOX pathways, may have some interesting properties in the treatment of OA. (J Rheumatol 2005;32:704–12)

Key Indexing Terms:
LEUKOTRIENES
OSTEOARTHRITIS

PROSTAGLANDINS

SYNOVIAL MEMBRANES
INTERLEUKIN 1

Osteoarthritis (OA) is the most common of arthritic diseases and is characterized by progressive loss of articular cartilage due to matrix degradation and repair mechanisms, as well as remodeling of the subchondral bone and formation of osteophytes¹⁻³. Synovitis is often observed and is considered to be secondary to the changes in the other tissues within the

joint. It is a key factor in the pathogenesis of the disease, and it appears to be fundamental to the appearance and progression of cartilage lesions⁴. Synovium has been reported to be one of the main sources for the production of proinflammatory mediators such as interleukin 1 β (IL-1 β) and tumor necrosis factor- α (TNF- α) that increase cartilage catabolism⁵⁻⁷. These cytokines stimulate the synthesis and release of lipid mediators derived from cyclooxygenase (COX) and lipoxygenase (LOX) metabolism of arachidonic acid (AA), such as prostaglandins (PG) and leukotrienes (LT), that are etiologically associated with arthritic and inflammatory diseases⁸⁻¹⁰.

Recent studies have focused more particularly on understanding the roles of LT. Studies in animal models as well as clinical trials have convincingly shown the potential of drugs that inhibit LOX-derived LT in the treatment of rheumatic diseases¹¹⁻¹³. LT play a major role in the inflammatory process¹⁴⁻¹⁶. They are synthesized via 5-LOX, an enzyme that requires the presence of the 5-LOX activating-protein (FLAP), in intact cells. Indeed, the activity of 5-LOX is stimulated by calcium in intact cells, whereas this is not required in *in vitro* experiments with broken cells¹⁷. The last

From the Osteoarthritis Research Unit, Hôpital Notre-Dame, Centre hospitalier de l'Université de Montréal, Montréal, Québec; Department of Orthopaedics, Hôpital Sacré-Coeur, Montréal, Québec; and Eberhard-Karls University, Tübingen, Germany.

Supported in part by a grant from Merckle GmbH, Ulm, Germany, and by la Chaire en Arthrose, Université de Montréal. P. Marcouiller is a recipient of the Fonds Marthe-Demers en gérontologie-gériatrie from the University of Montreal. Dr. Reboul is a recipient of the Dr. André Lanthier Scholarship from the University of Montreal.

P. Marcouiller, MSc; J-P. Pelletier, MD; M. Guévremont, MSc; J. Martel-Pelletier, PhD; P. Reboul, PhD, Osteoarthritis Research Unit, Hôpital Notre-Dame, Centre hospitalier de l'Université de Montréal; P. Ranger, MD, Department of Orthopaedics, Hôpital Sacré-Coeur; S. Laufer, PhD, Eberhard-Karls University.

Address reprint requests to Dr. P. Reboul, Unité de recherche en arthrose, CR-CHUM Centre hospitalier de l'Université de Montréal, 1560 rue Sherbrooke Est, Pavillon de Sève, Y-2604, Montréal, Québec H2L 4M1. E-mail: pascal.reboul@umontreal.ca

Submitted March 8, 2004; revision accepted November 10, 2004.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2005. All rights reserved.

biologically active metabolites of the 5-LOX cascade are leukotriene B₄ (LTB₄) and the so-called cysteinyl leukotrienes (LTC₄, LTD₄, and LTE₄), which are all derived from unstable intermediate LTA₄¹⁸. LTB₄ stimulates the production and release of proinflammatory cytokines from macrophages, lymphocytes, and, as recently discovered, from synovial membrane^{10,19}.

Lipoxins (LX, lipoxygenase interaction products) are yet another group of lipid mediators formed during AA metabolism. They are generated during the cellular interactions that occur as part of the multicellular host response to inflammation. LX are formed by transcellular metabolism from an intermediate derivative (5(6) epoxytetraene) that gives rise to the metabolically active products LXA₄, LXB₄, or 15(R)HETE for 15-epi-LX (aspirin-triggered LX). They are synthesized not only via the 5-LOX pathway but also by the action of 2 other enzymes, 12-LOX and 15-LOX^{20,21}. LX can be considered stop-signal mediators that possess anti-inflammatory effects. It has recently been shown that COX and LOX pathways can interact^{16,22}. Our group has reported that in human OA osteoblasts, NS398, a specific COX-2 inhibitor, can induce a shunt from the production of PGE₂ to LTB₄, thereby increasing the production of LTB₄²³.

We investigated the presence of an AA shunt in human OA synovial explants to delineate the mechanisms responsible for cross-talk between the COX and LOX pathways, and examined the influence of this shunt on the level of IL-1β synthesis. In addition, we studied the effect of pharmacological modulation of the COX and/or LOX pathways by drugs, including nonsteroidal antiinflammatory drugs (NSAID) with different inhibitory activities.

MATERIALS AND METHODS

Synovial membrane cultures. Human synovial membranes were obtained from 14 patients with OA (11 women, 4 men; mean age 66 ± 7 SD yrs) undergoing arthroplasty of the knee, who had been diagnosed based on American College of Rheumatology criteria²⁴. This study was approved by our institutional ethical and scientific committee. OA synovial membranes were aseptically dissected completely free from underlying fibrous and adipose tissue under a dissecting microscope, as described¹⁰. This study was based on previous work by our unit that established optimal experimental conditions to study synthesis of LTB₄ and cytokines such as IL-1β and TNF-α in human synovial explants¹⁰. Explant samples (~200 ± 50 mg, in duplicate) were divided randomly into experimental groups and incubated for 24–36 h (washout period) in explant culture medium [Dulbecco's modified Eagle's medium (HB-DMEM) + 10% fetal bovine serum (FBS) + Pen-Strep (100 U/100 μg)] at 37°C in a humidified atmosphere containing 5% CO₂/95% air prior to experimentation¹⁰. OA synovial membranes were incubated in the presence or absence of inhibitors of either the COX or LOX pathways in HB-DMEM containing 0.5% FBS and antibiotics during a period of either 72 h (eicosanoids and IL-1β measurements) or 24–72 h (RNA analysis). For eicosanoid measurements, ionomycin (Calbiochem-Novabiochem, La Jolla, CA, USA) at a final concentration of 20 nM was added 24 h before the end of incubation.

The effects of drugs acting at different levels of the arachidonic pathway [naproxen (Sigma), rofecoxib, NS398 (Calbiochem), licofelone (Merckle GmbH), nordihydro-guaiaretic acid (NDGA, Cayman Chemical), and FR 122047 (Tocris)] were investigated. Indeed, naproxen is an

inhibitor of both COX-1 and COX-2, while rofecoxib and NS398 are COX-2-specific inhibitors. Licofelone blocks COX-1 and COX-2 but also 5-LOX. NDGA is a broad inhibitor of 5-LOX, 12-LOX, and 15-LOX. FR 122047 is a COX-1-specific inhibitor.

Eicosanoids and IL-1β measurements. Measurements of PGE₂, LTB₄, [enzyme immunoassay (EIA), Cayman Chemical, Ann Arbor, MI, USA), LXA₄ (EIA, Lexington, KY, USA), and IL-1β (ELISA, R&D Systems, Minneapolis, MN, USA) in conditioned medium were performed according to the manufacturers' instructions.

Quantitative polymerase chain reaction (PCR). RNA extraction. OA synovial membranes were homogenized in Trizol reagent (Invitrogen, Burlington, ON, Canada) and total RNA were extracted, with some modifications, as described²⁵. Briefly, a first centrifugation was done before the addition of chloroform to eliminate debris. Then the supernatant was mixed with chloroform, cooled at 4°C for 5 min, and centrifuged (12,000 g, 10 min at 4°C). The aqueous phase was transferred to a new tube and precipitated with one volume of isopropyl alcohol overnight at -20°C. After centrifugation (12,000 g, 15 min at 4°C), the pellet was suspended in water. RNA was quantitated with RiboGreen® RNA quantitation reagent (Molecular Probes, Eugene, OR, USA).

RT-PCR. Two milligrams total RNA were used in the RT-PCR reaction. The assay was carried out in a Gene ATAQ controller (Amersham Pharmacia Biotech, Baie d'Urfé, QC, Canada). The RNA was converted into single-strand DNA with 50 units of M-MLV reverse transcriptase (Invitrogen) in 20 μl of reaction mixture containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 5 mM MgCl₂, 1 mM of each dNTP (Amersham Pharmacia Biotech), 2.5 μM random hexamers (Amersham), and 20 units RNA inhibitor. The mixture was incubated at 42°C for 45 min and at 99°C for 5 min. Real-time PCR on a Gene Amp 5700 sequence detector system (Applied Biosystems, Foster City, CA, USA) was performed in a total volume of 50 μl in the presence of 100 ng cDNA (samples, external standards, or water). MgCl₂ was added to a final concentration of 2 mM, each oligonucleotide primer added to a final concentration of 100 nM, and the SYBR® Green core reagents added according to the manufacturer's instructions (Applied Biosystems). To avoid sample contamination from previous amplifications, an initial step with AmpErase UNG was performed for 2 min at 50°C followed by AmpErase inactivation (10 min at 95°C). PCR was performed with an initial step for 5 min at 95°C followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. We verified that a single peak was obtained for each product using the Gene Amp 5700 system software. PCR primers for 5-LOX were sense 5'-CTG TTC CTG GGC ATG TAC CC and antisense 5'-GAC ATC TAT CAG TGG TCG TG. These primers produced a 467 bp amplified fragment. Specific primers for FLAP were sense 5'-TCT ACA CTG CCA ACC AGA AC and antisense 5'-ACG GAC ATG AGG AAC AGG, which gave a 223 bp amplified fragment. For GAPDH, used as a housekeeper gene, the sense primer was 5'-CAG AAC ATC ATC CCT GCC TCT, which corresponded to the 604–624 bp position; the antisense primer was 5'-GCT TGA CAA AGT GGT CGT TGA G and corresponded to the 901–922 position of the published sequence²⁶. Primers were synthesized by Medicorp (Montreal, QC, Canada).

Statistical analysis. Data are expressed as mean ± SEM. Mann-Whitney tests were performed to assess differences between groups, with results of *p* < 0.05 considered significant.

RESULTS

Previously it was shown that synovial explants produced LTB₄, which could subsequently regulate both IL-1β and TNF production^{10,27}. As cytokines, particularly IL-1β, are important for the development of OA, we investigated whether NSAID could regulate synovial membrane-produced LTB₄. Among the different drugs tested, only naproxen induced a dose-dependent increase in the production of

LTB₄ under unstimulated conditions, achieving a 5-fold increase (26.6 ± 5.2 pM, mean \pm SEM). None of the other drugs changed the basal levels of LTB₄ (Figure 1A). Moreover, incubation of synovial membranes with either 1 μ M of rofecoxib (COX-2-specific inhibitor) or with 1 μ M of FR 122047 (COX-1-specific inhibitor) did not modulate LTB₄ production under the same experimental conditions (data not shown). The apparent inefficacy of licofelone or NDGA at reducing the level of LTB₄ in the absence of naproxen could be explained by the extremely small amount of LTB₄ produced under unstimulated conditions. Indeed, the amounts found in the basal levels in the absence of naproxen were very close to the limit of detection of the assay. However, upon stimulation by LPS (1 μ g/ml), LTB₄ production in explants was significantly stimulated and both licofelone and NDGA inhibited the LPS-stimulated LTB₄

production, revealing the 5-LOX inhibitory activity of these compounds using our system (Figure 2A). Under stimulated conditions, naproxen did not enhance LTB₄ production. In basal conditions, the PGE₂ concentration produced by OA synovial explants was 1.9 ± 0.4 μ M. This production could be related in part to the COX-2 expression, as shown by results from immunoblotting. COX-2 expression was also found to be enhanced with LPS (data not shown). Under both basal and LPS-stimulated conditions, all NSAID inhibited PGE₂ production, while NDGA had no effect (Figures 1B, 2B). On the other hand, NS398 had no effect on LPS-stimulated LTB₄ production (Figure 2A).

As naproxen is a potent COX inhibitor, our results also suggested that PGE₂ could possibly regulate LTB₄ production. To investigate this, naproxen-treated synovial membranes were incubated with exogenous PGE₂ (1–100 nM).

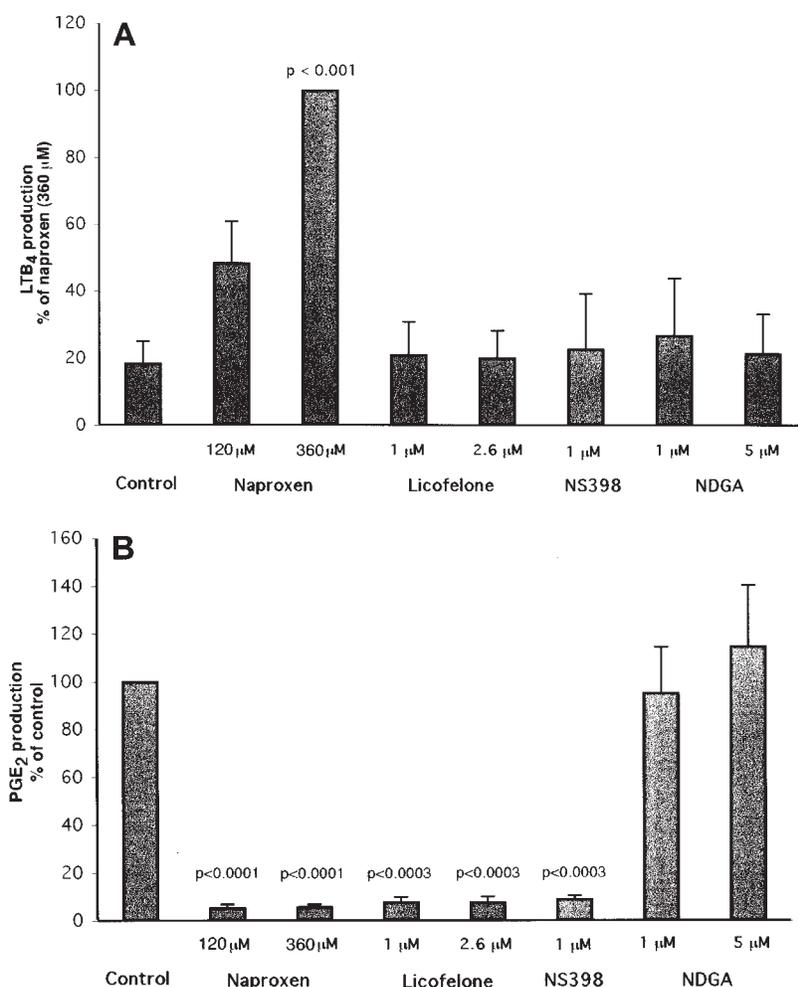


Figure 1. Production of LTB₄ (A) and PGE₂ (B) by human OA synovial membrane explants under unstimulated conditions, incubated for 72 h at 37°C in the absence (control) or presence of different NSAID (naproxen, a COX-1 and COX-2 inhibitor; licofelone, a COX-1, COX-2 and 5-LOX inhibitor; NS398, a COX-2-specific inhibitor; and NDGA, a 5-LOX, 12-LOX, and 15-LOX inhibitor). LTB₄ data were normalized to naproxen 360 μ M, whereas PGE₂ data were normalized to control. Values are mean \pm SEM from 6–8 independent experiments.

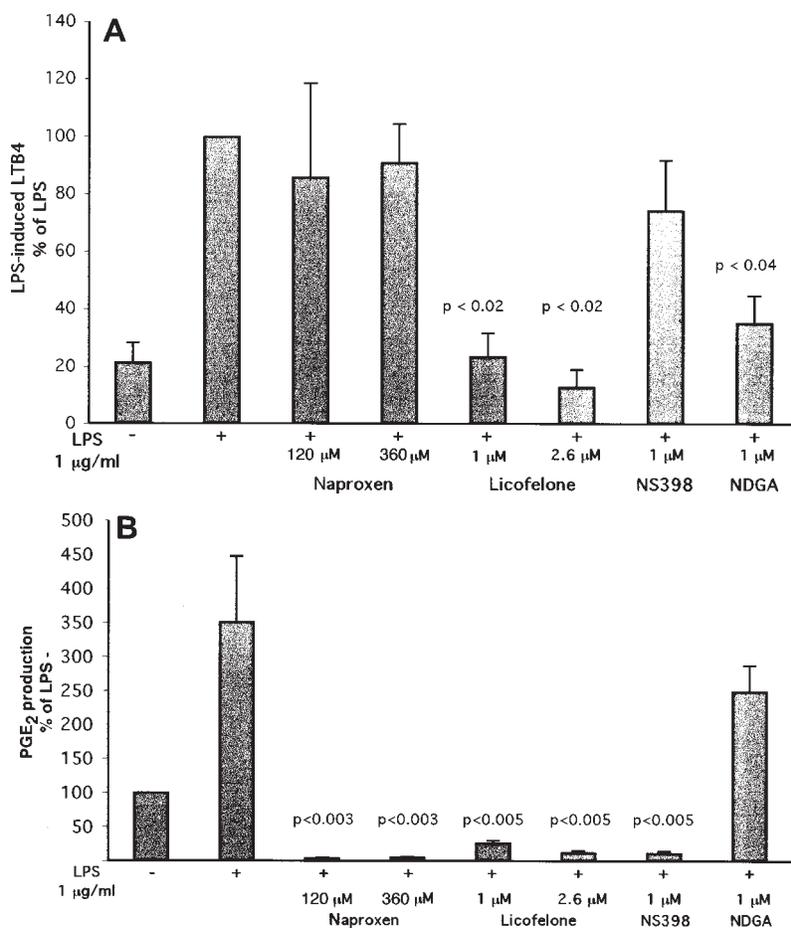


Figure 2. Production of LTB₄ (A) and PGE₂ (B) by human OA synovial membrane explants incubated 72 h at 37°C in the absence (-) or presence (+) of LPS (1 µg/ml). See Figure 1 legend for experimental conditions. LTB₄ data were normalized to LPS stimulation (LPS +) whereas PGE₂ data were normalized to control. Values are the mean ± SEM from 3–6 independent experiments.

The results showed that PGE₂ could partially inhibit the naproxen-induced increase of LTB₄ production. A PGE₂ concentration as low as 1 nM was effective and statistically significant, and a maximum of 50% of inhibition was reached with 10 nM of PGE₂ (Figure 3).

Further, we investigated whether naproxen and PGE₂ modified the expression of 5-LOX and FLAP, the 2 enzymes involved in LTB₄ production. These experiments were performed using real-time PCR, and the expression of 5-LOX and FLAP was measured at 24, 48, and 72 h. No variation from baseline in the expression of either 5-LOX or FLAP messengers was noted when synovial membranes were treated with naproxen alone or in combination with PGE₂ (data not shown).

Other LOX products, such as LX, have been reported to have antiinflammatory properties^{16,22}. Lipoxin A₄ has been shown to be implicated in the resolution of inflammation²⁸. Therefore, the following experiments were conducted to

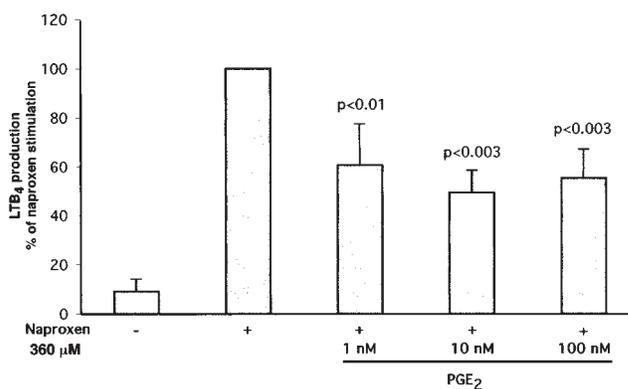


Figure 3. Reversibility of naproxen-induced LTB₄ production by PGE₂. Explants were incubated 72 h at 37°C in the absence (-) or presence (+) of naproxen 360 µM in combination with various concentrations of PGE₂ (1–100 nM). Results were normalized to naproxen-induced release of LTB₄. Values are the mean ± SEM from 4–6 independent experiments.

evaluate whether the shunt of AA to the LOX pathway might have an effect on production of LXA₄. Under unstimulated conditions, naproxen at a concentration that strongly stimulated LTB₄ production (360 μM) had no effect on LXA₄ levels (Figure 4A). In contrast, licofelone strongly inhibited LXA₄ synthesis. Under LPS stimulation, LXA₄ production was downregulated with naproxen and licofelone (Figure 4B). When we calculated the LTB₄/LXA₄ ratio under either unstimulated (Figure 5A) or LPS-stimulated (Figure 5B) conditions, our results showed an increase of this ratio with naproxen, which may induce some conditions favoring an inflammatory process. For its part, licofelone kept the LTB₄/LXA₄ ratio to the control level.

As LTB₄ was previously shown to stimulate cytokine synthesis, we investigated whether naproxen could upregulate IL-1β synthesis. Under unstimulated conditions, the IL-1β level was about 4 ± 2.2 nM, and no increase in IL-1β synthesis was noted with naproxen (data not shown). However, under LPS-stimulated conditions, both naproxen concentrations induced an overproduction of IL-1β, whereas licofelone had no effect (Figure 6).

DISCUSSION

In our study, we demonstrated the capacity of NSAID that inhibit both COX-1 and COX-2 to induce a shunt of AA

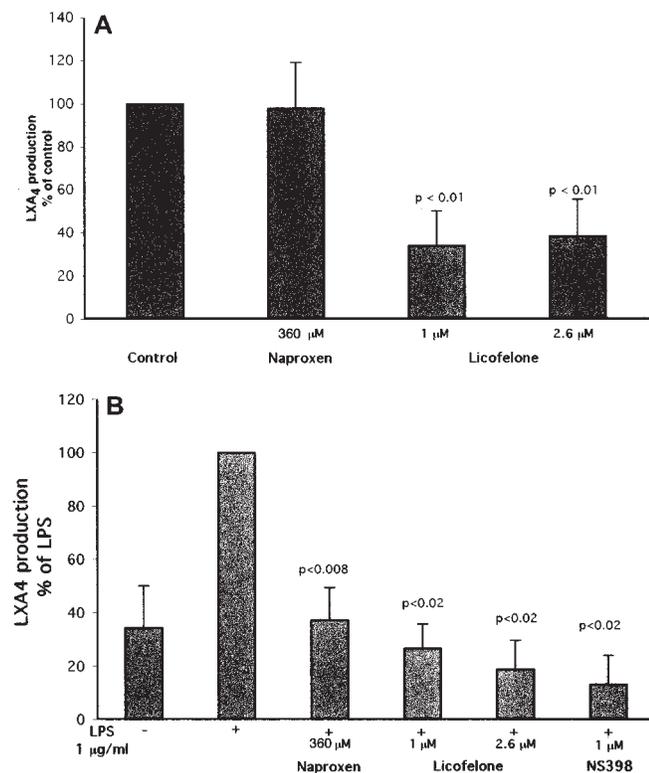


Figure 4. Production of LXA₄ by human OA synovial membrane explants under unstimulated (A) or LPS-stimulated conditions (B). Explants were incubated 72 h at 37°C in the absence (-) or presence (+) of LPS (1 μg/ml). Results were normalized to the control (A) or to LPS stimulation (LPS +) (B). Values are the mean ± SEM from 4–6 independent experiments.

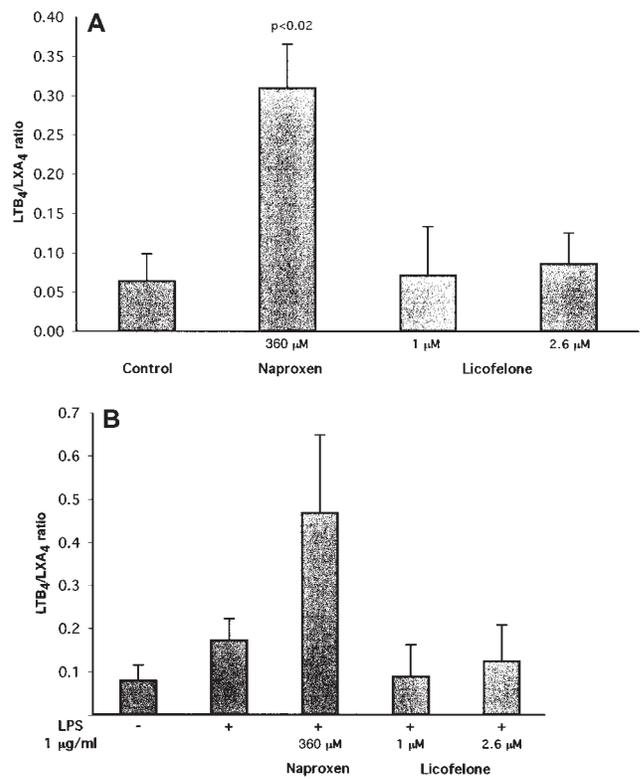


Figure 5. Ratio of LTB₄/LXA₄ production by human OA synovial membrane explants under unstimulated (A) or LPS-stimulated conditions (B). Explants were incubated 72 h at 37°C in the absence (-) of LPS (1 μg/ml) or coincubated with LPS (+) and either naproxen or licofelone. Values are the mean ± SEM from 4–5 independent experiments.

from the COX to the LOX pathway in OA synovial membranes. This shunting phenomenon was associated with an upregulation in IL-1β synthesis. The regulation of this shunt may be at the posttranscriptional or posttransductional level of 5-LOX and FLAP. The addition of exogenous PGE₂ significantly reversed naproxen's effect. By contrast, specific COX-2 inhibitors (rofecoxib, NS398) did not induce such a shunt. Using NDGA, a 5-, 12-, and 15-LOX inhibitor, and measuring PGE₂ levels, we found that a shunt of AA from the LOX to the COX pathway did not exist in synovial membranes. Moreover, treatment with naproxen increased the LTB₄/LXA₄ ratio, which may favor the inflammation process. Unlike naproxen and as expected, licofelone, a dual inhibitor of COX and LOX pathways, decreased levels of PGE₂, LTB₄, and lipoxins and prevented the overexpression of IL-1β under LPS stimulation.

The finding that LPS-stimulated synovial explants did not produce higher levels of LTB₄ in the presence of naproxen could be explained by the fact that maximum production was already achieved, likely because of the limited amount of 5-LOX, which concurs with the concept that LTA₄ limits 5-LOX activity^{29,30}. Indeed, a previous study showed that 5-LOX produced LTA₄, which in return covalently bound and dose-dependently inactivated 5-LOX. It

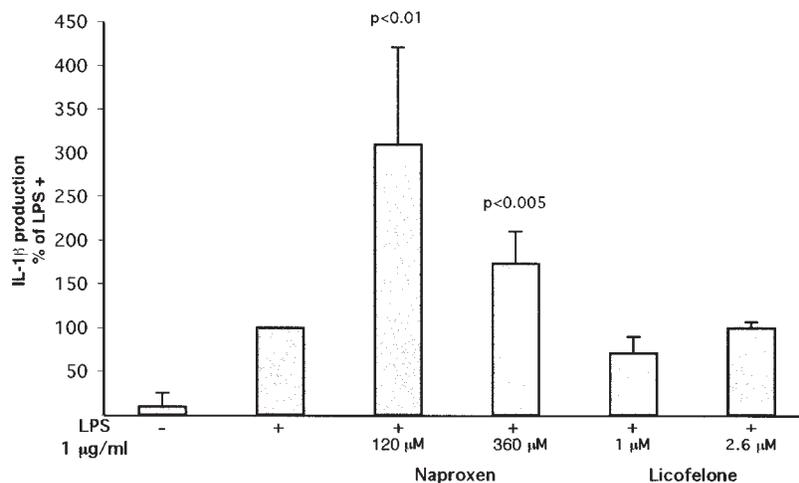


Figure 6. Production of IL-1 β by human OA synovial membrane explants under LPS-stimulated conditions. Explants were incubated 72 h at 37°C in the absence (-) of LPS (1 μ g/ml) or coincubated with LPS (+) and either naproxen or licofelone. Results were normalized to LPS stimulation (LPS +). Values are the mean \pm SEM from 3–6 independent experiments.

has been estimated that each enzyme can catalyze approximately 500 reactions before becoming inactive^{29,30}. The LTB₄ level obtained in this study, however, is 10 to 100 times lower than that found by He, *et al*¹⁰. The difference could be due to the ionophores used. Indeed, we chose to use only ionomycin, whereas He, *et al* used the combination of ionomycin and thapsigargin¹⁰, a combination that may have mobilized a higher level of intracellular Ca²⁺ and activated both nuclear and cytosolic 5-LOX, since 5-LOX has been observed in cytosol and nuclei. It is possible that ionomycin alone induced only the activity of the cytosolic 5-LOX³¹. Nevertheless, the level of LTB₄ production we observed concurs with that previously found in arthritis synovial fluids³². The naproxen-induced LTB₄ production was regulated at the posttranscriptional level since neither 5-LOX nor FLAP mRNA levels were modulated. In general, the regulation of 5-LOX and FLAP genes appears similar, reflecting the dual requirement for LTB₄ production³³. However, exceptions to this coordinated expression exist. For example, differentiation of HL-60 cells into granulocyte-like cells with DMSO results in a concurrent increase in 5-LOX and FLAP mRNA, while differentiation with phorbol esters toward a macrophage-like cell line results in an increase of FLAP but not in 5-LOX³⁴. From our model, it can be hypothesized that 5-LOX and FLAP messengers might be regulated at a posttranscriptional level. Indeed, it was reported that 5-LOX expression was complex and also involved control mechanisms for mRNA stability, mRNA translation, enzyme translocation, substrate availability, cofactor availability, and enzyme activation³¹. Moreover, LTB₄ synthase, another enzyme involved in LTB₄ production, could be regulated as well. The presence of several different cell types in the synovial membrane increases the difficulty in discovering precisely which enzyme and which

level of regulation was involved. These several possibilities raise a complex question, which is currently under investigation in our laboratory.

PGE₂ was found to partially downregulate naproxen-induced LTB₄ production. It is important to note that in this study, the PGE₂ concentration found in the OA synovium was 0.5 to 3 μ M, and therefore the exogenous concentrations of PGE₂ added were still below pathological concentrations. The PGE₂ effect might occur through the inhibition of 5-LOX activity. Levy, *et al*²⁸ recently showed that PGE₂ inhibited LTB₄ production in human polymorphonuclear cells, and that this production could be rescued with the addition of LTA₄, indicating that inhibition of LTB₄ biosynthesis by PGE₂ occurred via 5-LOX inactivation. They also observed that the inhibition did not result from inactivation of either cytosolic phospholipase A₂ or LTA₄ hydrolase²⁸. Nevertheless, it seems likely that another pathway, not yet identified, regulating LTB₄ production may exist in our synovial explant culture model under treatment with naproxen. Among the different possibilities, one could suggest that naproxen acts via the peroxisome proliferator-activated receptor (PPAR) system. Since PPAR- γ isoform is very sensitive to activation by naproxen (10 μ M)³⁵, the concentration of the drug used in our study could have activated PPAR.

The results we obtained with NS398 are in certain aspects similar to and in other aspects different from previous reports. On one hand, NS398 under LPS stimulation confirms the findings of an absence in decrease in LTB₄ production, as described by He, *et al*¹⁰. On the other hand, NS398 did not induce the shunt that was previously reported in osteoblasts²³. The latter results may be explained by a differential role played by the different COX. Studies on COX-2-specific inhibitors have shown that COX-2 activity

is of utmost importance in the homeostasis of bone under normal conditions^{36,37}. Further, throughout our study we did not observe a shunt in the presence of a COX-1-specific inhibitor alone (FR 122047). These results support the concept that only a complete suppression of PGE₂ production by dual inhibition of COX with naproxen treatment could induce the shunt. This is supported by the experiments showing that even very low concentration of exogenous PGE₂ (1–10 nM) could at least partially reverse the shunt. This finding also corroborates the important role of COX-1 activity in regulating the AA pool in synovial membranes under unstimulated conditions.

Conversion into lipoxins, and particularly into LXA₄ and LXB₄, in mammals is another metabolic route for AA. Lipoxins are generated during cell-cell interactions and serve as braking signals of inflammation^{21,38}. We observed that under basal conditions naproxen had no effect on LXA₄ production. In contrast, naproxen inhibited the LPS-induced LXA₄ production, suggesting an obvious differential regulation of LXA₄ under both basal and stimulated conditions. Indeed, it is well known that LPS activates numerous intracellular signaling routes that could be PGE₂-dependent. Although these findings are speculative, one might suggest that under LPS-stimulated conditions the increase of LXA₄ production was related to an upregulation of 15-LOX expression by PGE₂, as reported by Levy, *et al*²⁸. This finding is also supported by the experiments showing that LXA₄ production was also inhibited by NS398 under these conditions. Perhaps the most relevant finding was that the LTB₄/LXA₄ ratio increased with naproxen, an increase that could cause an imbalance in the response of cells to each component. This imbalance is of particular importance in joint diseases, since LXA₄ has been shown to inhibit IL-1 β -induced IL-6, IL-8, and metalloproteinase-3 production in

human synovial fibroblasts and enhance synthesis of tissue inhibitors of metalloproteinases³⁹. In summary, it could be hypothesized that in the absence of inflammation, the intake of naproxen may lead to an overproduction of LTB₄, which would represent an environmental condition that may favor an inflammatory process in the joint (Figure 7). In the presence of inflammation, the intake of naproxen would downregulate LXA₄ production and increase the LTB₄/LXA₄ ratio, which would create an environment favorable to sustaining a proinflammatory condition.

Studies have shown that LTB₄ can modulate the synthesis of IL-1 β in synovial explants^{10,19,40}. Thus it seemed relevant to investigate whether upregulation of LTB₄ induced by naproxen affected the level of IL-1 β synthesis. Indeed, IL-1 β synthesis increased only with coincubation of naproxen and LPS. Although 2 preceding studies have shown that LTB₄ can induce synthesis of IL-1 β ^{10,40}, the concentrations used were high (10⁻⁹ and 10⁻⁸ M) and could have recruited other signaling pathways also present when the synovium was stimulated with LPS. Moreover, IL-1 production could be regulated by transcriptional, posttranscriptional (mRNA stability), translational, and posttranslational (protein maturation) mechanisms. Under unstimulated conditions, LTB₄ alone could not stimulate IL-1 synthesis. In contrast, under LPS stimulation, which mimics inflammatory conditions and activates numerous signaling pathways implicated in the different steps of IL-1 β production. Therefore it seems that LTB₄ alone is not potent enough to induce IL-1 β , but instead acts as a cofactor of one of these pathways, thus enhancing IL-1 production. Using MK886 (a 5-LOX inhibitor) alone or in combination with LTB₄, Rainsford, *et al* demonstrated that IL-1 β synthesis in synovial membranes was regulated by LTB₄¹⁹. However, this regulation was only possible with inflamed membranes. These investigators also

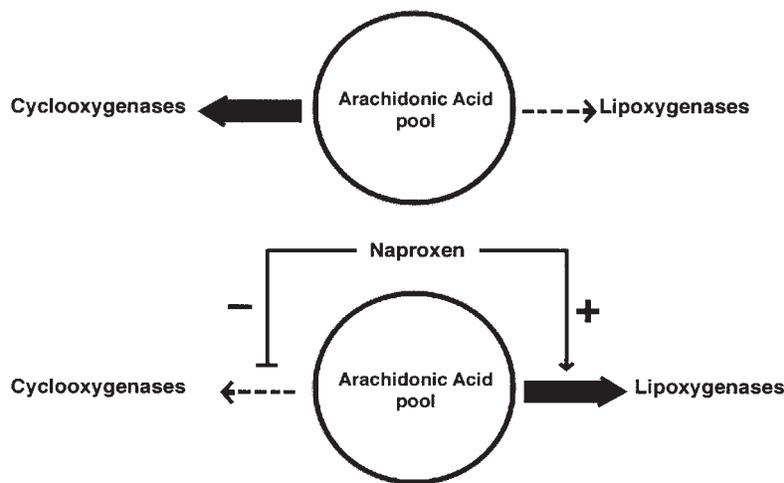


Figure 7. The arachidonic acid (AA) shunt in the presence of naproxen. In the absence of naproxen, free AA is mainly used by the COX pathway. Conversely, by inhibiting the COX and by an unidentified pathway, naproxen directs free AA to the LOX pathway.

hypothesized that the reported lack of effect of MK886 on monocyte IL-1 production⁴² may have been due to the lack of an inflamed state of those cells compared to those from synovial membrane samples. IL-1 is not the only cytokine that is differently regulated by naproxen under different stimulatory conditions. Indeed, naproxen has no effect on peripheral blood mononuclear cell IL-6 production under unstimulated conditions, whereas it increases the IL-6 synthesis under LPS stimulation⁴³. This was not the first time that eicosanoids such as LTB₄ have been found to have differential effects under basal and stimulated conditions. Faour, et al showed that PGE₂ and LTB₄ can reverse NS398-dependent inhibition of IL-1β-induced COX-2 expression, but have no effect in quiescent unstimulated synovial fibroblasts⁴⁴. Another aspect of LPS stimulation was reported by Jiang, *et al*, who argue that the LPS stimulation pathway may overcome the PPAR pathway in OA synovial membranes⁴⁵. Overall, as the main difference between the effect of naproxen and licofelone relates to the capacity for inhibiting LTB₄ production, these findings suggest that under specific experimental conditions LTB₄ is an important cofactor in the synthesis of IL-1β in OA synovium. Moreover, naproxen is well known to be an inhibitor of cartilage matrix synthesis, while it is unable to inhibit proteoglycanase activity^{46,47}. One may speculate that either LTB₄ or IL-1β, which have the same properties toward the matrix, could be involved in this process.

Our study shows that the COX pathways generally metabolize AA in OA synovial explants. However, in certain circumstances of PGE₂ depletion provoked by drugs that inhibit both COX-1 and COX-2 enzymes, a shunt occurs from the COX to the LOX pathway. This phenomenon induces excess production of LTB₄ as well as an increase in the LTB₄/LXA₄ ratio, and leads to increased production of IL-1β, a cytokine known to play a major role in the pathophysiology of OA. Simultaneously controlling both PGE₂ and LTB₄ production, licofelone is an interesting new drug that may present advantages for the treatment of OA because of its capacity to inhibit this shunt.

ACKNOWLEDGMENT

The authors thank Lorraine Doré, Santa Fiori, and Julie Francoeur for their assistance in manuscript preparation.

REFERENCES

- Pelletier JP, Martel-Pelletier J, Howell DS. Etiopathogenesis of osteoarthritis. In: Koopman WJ, editor. Arthritis and allied conditions. A textbook of rheumatology. Baltimore: Williams & Wilkins; 2001:2195-245.
- Lohmander LS. Articular cartilage and osteoarthritis. The role of molecular markers to monitor breakdown, repair and disease. *J Anat* 1994;184:477-92.
- Hamerman D. The biology of osteoarthritis. *N Engl J Med* 1989;320:1322-30.
- Recklies AD, Poole AR, Banerjee S, et al. Pathophysiologic aspects of inflammation in diarthroidal joints. In: Buckwalter JA, editor. Orthopaedic basic science: biology and biomechanics of the musculoskeletal system. Rosemont, IL: American Academy of Orthopaedic Surgeons; 2000:489-530.
- Brennan FM, Chantry D, Jackson AM, Maini RN, Feldmann M. Cytokine production in culture by cells isolated from the synovial membrane. *J Autoimmun* 1989;2 Suppl:177-86.
- Elson CJ, Mortuza FY, Perry MJ, Warnock MG, Webb GR, Westacott CI. Cytokines and focal loss of cartilage in osteoarthritis. *Br J Rheumatol* 1998;37:106-7.
- Martel-Pelletier J, Mineau F, Jolicoeur FC, Cloutier JM, Pelletier JP. In vitro effects of diacerhein and rhein on IL-1 and TNF-α systems in human osteoarthritic tissues. *J Rheumatol* 1998;25:753-62.
- Wittenberg RH, Willburger RE, Kleemeyer KS, Peskar BA. In vitro release of prostaglandins and leukotrienes from synovial tissue, cartilage, and bone in degenerative joint diseases. *Arthritis Rheum* 1993;36:1444-50.
- Moilanen E, Alanko J, Nissila M, Hamalainen M, Isomaki H, Vapaatalo H. Eicosanoid production in rheumatoid synovitis. *Agents Actions* 1989;28:290-7.
- He W, Pelletier JP, Martel-Pelletier J, Laufer S, Di Battista JA. The synthesis of interleukin-1β, tumour necrosis factor-α and interstitial collagenase (MMP-1) is eicosanoid dependent in human OA synovial membrane explants: Interactions with anti-inflammatory cytokines. *J Rheumatol* 2002;29:546-53.
- Musser JH, Kreft AF. 5-lipoxygenase: properties, pharmacology, and the quinoliny(bridged)aryl class of inhibitors. *J Med Chem* 1992;35:2501-24.
- Laufer SA, Augustin J, Dannhardt G, Kiefer W. (6,7-Diaryldihydropyrrolizin-5-yl)acetic acids, a novel class of potent dual inhibitors of both cyclooxygenase and 5-lipoxygenase. *J Med Chem* 1994;37:1894-7.
- Laufer S, Tries S, Augustin J, Dannhardt G. Pharmacological profile of a new pyrrolizine derivative inhibiting the enzymes cyclo-oxygenase and 5-lipoxygenase. *Arzneimittelforschung* 1994;44:629-36.
- Sala A, Zarini S, Bolla M. Leukotrienes: lipid bioeffectors of inflammatory reactions. *Biochemistry Mosc* 1998;63:84-92.
- Bray MA, Ford-Hutchinson AW, Smith MJ. Leukotriene B₄: an inflammatory mediator in vivo. *Prostaglandins* 1981;22:213-22.
- Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* 2001;294:1871-5.
- Radmark OP. The molecular biology and regulation of 5-lipoxygenase. *Am J Respir Crit Care Med* 2000;161:S11-5.
- Penrose JF, Austen KF, Lam BK. Leukotrienes: biosynthetic pathways, release and receptor-mediated actions with relevance to disease states. In: Gallin JL, Snyderman R, editors. Inflammation: basic principles and clinical correlates. Philadelphia: Lippincott Williams & Wilkins; 1999:361-72.
- Rainsford KD, Ying C, Smith F. Effects of 5-lipoxygenase inhibitors on interleukin production by human synovial tissues in organ culture: comparison with interleukin-1-synthesis inhibitors. *J Pharm Pharmacol* 1996;48:46-52.
- Serhan CN, Levy BD, Clish CB, Gronert K, Chiang N. Lipoxins, aspirin-triggered 15-epi-lipoxin stable analogs and their receptors in anti-inflammation: a window for therapeutic opportunity. *Ernst Schering Res Found Workshop* 2000;31:143-85.
- Serhan CN. Lipoxins and novel aspirin-triggered 15-epi-lipoxins (ATL): a jungle of cell-cell interactions or a therapeutic opportunity? *Prostaglandins* 1997;53:107-37.
- Celotti F, Laufer S. Anti-inflammatory drugs: new multitarget compounds to face an old problem. The dual inhibition concept. *Pharmacol Res* 2001;43:429-36.
- Paredes Y, Massicotte F, Pelletier JP, Martel-Pelletier J, Laufer S, Lajeunesse D. Study of role of leukotriene B₄ in abnormal function

- of human subchondral osteoarthritis osteoblasts. Effects of cyclooxygenase and/or 5-lipoxygenase inhibition. *Arthritis Rheum* 2002;46:1804-12.
24. Altman RD, Asch E, Bloch DA, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. *Arthritis Rheum* 1986;29:1039-49.
 25. Tardif G, Di Battista JA, Pelletier J-P, Martel-Pelletier J. Measurement of interleukin mRNA by Northern blotting using a non-radioactive detection method. In: O'Neill L, editor. *Interleukin protocols*. Totowa, NJ: Humana Press Inc.; 2000.
 26. Martel-Pelletier J, Mineau F, Tardif G, et al. Tenidap reduces the level of interleukin-1 receptors and collagenase expression in human arthritic synovial fibroblasts. *J Rheumatol* 1996;23:24-31.
 27. Bonnet C, Bertin P, Cook-Moreau J, Chable-Rabinovitch H, Treves R, Rigaud M. Lipoxygenase products and expression of 5-lipoxygenase and 5-lipoxygenase-activating protein in human cultured synovial cells. *Prostaglandins* 1995;50:127-35.
 28. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class switching during acute inflammation: signals in resolution. *Nat Immunol* 2001;2:612-9.
 29. Schewe T, Kuhn H, Rapoport SM. Positional specificity of lipoxygenases and their suitability for testing potential drugs. *Prostaglandins Leukot Med* 1986;23:155-60.
 30. Rouzer CA, Samuelsson B. Leukocyte arachidonate 5-lipoxygenase: isolation and characterization. *Methods Enzymol* 1990;187:312-9.
 31. Silverman ES, Drazen JM. The biology of 5-lipoxygenase: function, structure, and regulatory mechanisms. *Proc Assoc Am Physicians* 1999;111:525-36.
 32. Sahap Atik O. Leukotriene B4 and prostaglandin E2-like activity in synovial fluid in osteoarthritis. *Prostaglandins Leukot Essent Fatty Acids* 1990;39:253-354.
 33. Reid GK, Kargman S, Vickers PJ, et al. Correlation between expression of 5-lipoxygenase-activating protein, 5-lipoxygenase, and cellular leukotriene synthesis. *J Biol Chem* 1990;265:19818-23.
 34. Bennett CF, Chiang MY, Monia BP, Crooke ST. Regulation of 5-lipoxygenase and 5-lipoxygenase-activating protein expression in HL-60 cells. *Biochem J* 1993;289:33-9.
 35. Jaradat MS, Wongsud B, Phornchirasilp S, et al. Activation of peroxisome proliferator-activated receptor isoforms and inhibition of prostaglandin H(2) synthases by ibuprofen, naproxen, and indomethacin. *Biochem Pharmacol* 2001;62:1587-95.
 36. Zhang X, Schwarz EM, Young DA, Puzas JE, Rosier RN, O'Keefe RJ. Cyclooxygenase-2 regulates mesenchymal cell differentiation into the osteoblast lineage and is critically involved in bone repair. *J Clin Invest* 2002;109:1405-15.
 37. Zhang X, Morham SG, Langenbach R, et al. Evidence for a direct role of cyclo-oxygenase 2 in implant wear debris-induced osteolysis. *J Bone Miner Res* 2001;16:660-70.
 38. Brady HR, Serhan CN. Lipoxins: putative braking signals in host defense, inflammation and hypersensitivity. *Curr Opin Nephrol Hypertens* 1996;5:20-7.
 39. Sodin-Semrl S, Taddeo B, Tseng D, Varga J, Fiore S. Lipoxin A₄ inhibits IL-1 beta-induced IL-6, IL-8, and matrix metalloproteinase-3 production in human synovial fibroblasts and enhances synthesis of tissue inhibitors of metalloproteinases. *J Immunol* 2000;164:2660-6.
 40. Kageyama Y, Koide Y, Miyamoto S, Yoshida TO, Inoue T. Leukotrien B4-induced interleukin-1β in synovial cells from patients with rheumatoid arthritis. *Scand J Rheumatol* 1994;23:148-50.
 41. Prete PE, Gurakar-Osborne A. The contribution of synovial fluid lipoproteins to the chronic synovitis of rheumatoid arthritis. *Prostaglandins* 1997;54:689-98.
 42. Hoffman T, Lee YL, Lizzio EF, et al. Absence of modulation of monokine production via endogenous cyclooxygenase or 5-lipoxygenase metabolites: MK-886 (3-[1-(4-chlorobenzyl)-3-t-butyl-thio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid), indomethacin, or arachidonate fail to alter immunoreactive interleukin-1 beta, or TNF-alpha production by human monocytes in vitro. *Clin Immunol Immunopathol* 1991;58:399-408.
 43. Sipe JD, Bartle LM, Loose LD. Modification of proinflammatory cytokine production by the antirheumatic agents tenidap and naproxen. A possible correlate with clinical acute phase response. *J Immunol* 1992;148:480-4.
 44. Faour WH, He Y, He QW, et al. Prostaglandin E2 regulates the level and stability of cyclooxygenase-2 mRNA through activation of p38 mitogen-activated protein kinase in interleukin-1 beta treated synovial fibroblasts. *J Biol Chem* 2001;276:31720-31.
 45. Jiang C, Ting AT, Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature* 1998;391:82-6.
 46. Arner EC, Hughes CE, Decicco CP, Caterson B, Tortorella MD. Cytokine-induced cartilage proteoglycan degradation is mediated by aggrecanase. *Osteoarthritis Cartilage* 1998;6:214-28.
 47. Dingle JT, Parker M. NSAID stimulation of human cartilage matrix synthesis — A study of the mechanism of action of aceclofenac. *Clin Drug Invest* 1997;14:353-62.