

New Insights into Prostaglandin Biology



Prostaglandins (PG) are critical modulators of numerous physiological and pathophysiological conditions including inflammation, immune regulation, cancer, and arthritis. Regulation of their production continues to be the subject of intensive investigation. Cyclooxygenases (COX) are the enzymes catalyzing the rate-limiting step in PG synthesis, converting arachidonic acid into PGH_2 . Three isoforms of this enzyme have been identified (COX-1, COX-2, COX-3). COX-1 is expressed constitutively in most tissues and is believed to generate PG for physiologic functions such as regulating vascular homeostasis, protecting gastric mucosa, and maintaining renal integrity. COX-2, by contrast, is almost undetectable under physiologic conditions in most tissues. However, its expression is upregulated by proinflammatory stimuli, growth factors, and mitogens and is involved in pathologic conditions, notably inflammation¹. Recently, a third isoform, COX-3, was cloned and was shown to share the catalytic features of COX-1 and COX-2². In the past decade, significant advances have been made in understanding the role of the COX enzymes in many biological processes. In addition, studies involving the pharmacological inhibition of PG production have generated encouraging results. Several selective or preferential COX-2 inhibitors have been developed and were shown to be effective in clinical trials. It is reasonable to expect that a greater understanding of the regulation of PG production will result in the development of more effective strategies in the treatment of arthritis.

In many cells, the main PG species produced is PGE_2 . *In vivo* studies have shown that COX-2 inhibitors reduce PGE_2 synthesis more profoundly than other PG³. In addition, studies with a specific monoclonal antibody to PGE_2 indicated that the major PG that contributes to inflammation is PGE_2 ⁴. However, the identity of the enzymes responsible for the isomerization of PGH_2 to PGE_2 has been a mystery.

PROSTAGLANDIN E SYNTHASES

Recently, 2 PGE_2 synthases (PGES) were cloned and were shown to possess high and specific PGH_2 to PGE_2 converting activity⁵⁻⁷. One PGES isoform is constitutively expressed in a wide variety of cells and tissues while the other isoform is inducible. The constitutive PGES is detected in the cytosol (cPGES) and is unresponsive to

proinflammatory cytokines and bacterial lipopolysaccharides (LPS)⁶. The inducible isoform is located on the perinuclear membrane (mPGES) and is induced by proinflammatory stimuli, and this induction is decreased by glucocorticoids^{5,7}. Thoren and Jakobsson⁸ showed that mPGES is regulated in a coordinated manner with COX-2 in the human lung cell line A549, suggesting the existence of a potential link between both enzymes. Indeed, coexpression experiments revealed that mPGES is preferentially linked with COX-2, promoting induced immediate PGE_2 production, while cPGES is functionally linked to COX-1 and may contribute to production of PGE_2 required for the maintenance of tissue homeostasis^{6,7}. Recently, Mancini, *et al*⁹ found that 5 days after rat treatment with adjuvant, inducible PGES expression was increased in the lung and the adjuvant-treated paw, while no PGES was detected in the naive (vehicle-treated) rat paw. Inducible PGES expression was accompanied by an upregulation of COX-2 mRNA and protein expression as compared with the naive paw. Stichtenoth, *et al*¹⁰ examined the expression of PGES in RA synovial fibroblasts and found that both cPGES and mPGES are present in these cells, but only mPGES expression is modulated. In these cells, while very low concentrations were detected under basal conditions, interleukin 1 β (IL-1 β) or tumor necrosis factor- α (TNF- α) treatment resulted in a marked increase in mPGES mRNA expression. Further, dexamethasone dose-dependently inhibits IL-1 β -induced mPGES expression. It is likely that a better understanding of the expression and activity of the inducible PGES may help to develop more effective therapies for diseases associated with an increase in PGE_2 synthesis, such as inflammatory arthropathies.

15-deoxy- $\Delta^{12,14}$ -PGJ₂

Recently, several studies showed that 15-deoxy- $\Delta^{12,14}$ -PGJ₂ (15d-PGJ₂), the end product metabolite of PGD₂, displays antiinflammatory properties¹¹. 15d-PGJ₂ induces its effects possibly through binding to the peroxisome proliferator-activated receptor γ (PPAR γ). The PPAR are a family of ligand-activated transcription factors, which heterodimerize with retinoic X receptor (RXR) after ligand binding. This complex binds to PPAR-responsive elements (PPRE) in the promoter regions of target genes. PPAR γ was originally

identified because of its role in adipocyte differentiation, glucose metabolism, and lipid uptake. More recently, several studies have shown that PPAR γ is expressed in many inflammatory and immune cells including macrophages, T lymphocytes, B lymphocytes, and osteoclasts¹¹.

Our laboratory recently investigated whether PPAR γ is expressed and transcriptionally active in human articular chondrocytes and synovial fibroblasts. Using reverse transcription-polymerase chain reaction and immunohistochemistry, we showed that PPAR γ is expressed and transcriptionally active in both cell types^{12,13}. Treatment of human chondrocytes with 15d-PGJ₂ or a synthetic PPAR γ ligand (BRL 49653) resulted in inhibition of IL-1 β -induced nitric oxide (NO) and collagenase-3 or metalloprotease-13 (MMP-13) production¹². Similarly, PPAR γ activators suppress IL-1 β -induced collagenase-1 (MMP-1) expression and production in human osteoarthritis synovial fibroblasts¹³ and IL-1 β and TNF- α expression in rheumatoid synovial fibroblasts¹⁴. Rat synovial fibroblasts also express PPAR γ , and 15d-PGJ₂ dose-dependently prevents LPS-induced iNOS, COX-2, IL-1, and TNF- α expression¹⁵. The inhibitory effect of 15d-PGJ₂ takes place at the transcriptional level, is at least in part mediated through PPAR γ , and involves the inhibition of the transcription factors AP-1 and NF- κ B^{12,16}. 15d-PGJ₂ was also shown to be protective in an animal model of arthritis. Indeed, Kawahito, *et al*¹⁷ showed that intraperitoneal administration of 15d-PGJ₂ ameliorates the chronic inflammation of adjuvant-induced arthritis with suppression of pannus formation and mononuclear cell infiltration in female Lewis rats. Together, these results suggest that modulation of 15d-PGJ₂ production may constitute a therapeutic target in the treatment of rheumatic diseases.

The effect of PPAR ligands, including 15d-PGJ₂, on COX-2 expression was examined in several cell systems. PPAR ligands were shown to prevent the induction of COX-2 expression in human aortic smooth muscle cells¹⁸, human epithelial cells¹⁹, macrophage-like differentiated U937 cells²⁰, human primary monocytes²¹, and human astrocytes²². However, PPAR ligands were also reported to induce COX-2 expression in several cell types including epithelial cells²³, rabbit corneal epithelial cells²⁴, and human synovial fibroblasts²⁵. Thus regulation of COX-2 expression by PPAR is likely cell-type-specific and may depend on the local environment.

In human chondrocytes, we found that 15d-PGJ₂ prevented IL-1 β -induced COX-2 expression and PGE₂ production²⁶. This was consistent with 2 studies showing that 15d-PGJ₂ inhibits LPS- and IL-1 β -induced COX-2 expression in rat and human rheumatoid synovial fibroblasts^{15,27}. Interestingly, when 15d-PGJ₂ was used alone, it induced COX-2 expression without concomitant elevation in PGE₂ production. Therefore, 15d-PGJ₂ appears to have opposite regulatory effects on COX-2; it promotes COX-2 expression when used alone but prevents it in the presence

of inducers. It is possible that the activity of COX-2 enzyme induced by 15d-PGJ₂ is directed towards the synthesis of antiinflammatory PG. In accord with this, it has been reported that the resolution of inflammation in carrageenan-induced pleurisy in rats is associated with an increase in PGD₂ and 15d-PGJ₂ synthesis, while the production of PGE₂ is diminished²⁸.

ARE THERE ADDITIONAL CYCLOOXYGENASES?

It is likely that the protein induced by 15d-PGJ₂ represents an additional COX enzyme that, unlike COX-1 and COX-2, does not produce PGE₂. Flower and Vane²⁹ postulated the existence of additional COX enzymes 30 years ago. These authors analyzed the effect of acetaminophen, which, unlike nonsteroidal antiinflammatory drugs (NSAID), produces analgesia and antipyresis but has little effect on inflammation. They found that acetaminophen was more potent in inhibiting PG synthesis by the rabbit and dog brain than by the spleen. The expression of a third COX enzyme was also reported in cultured J774.2 macrophages by Simmons, *et al*³⁰. This isoform was induced by high concentrations of NSAID and appears to be a variant of COX-2 that remains free in the cytosol rather than membrane-bound. The activity of this COX-2-like form was more sensitive to inhibition with acetaminophen than the LPS-induced COX-2³⁰. More recently, a COX-3 and two COX-1-like proteins were described, and the 3 isoforms were shown to be COX-1 variants². In contrast to the COX-1-like proteins, the COX-3 shares the catalytic features of COX-1 and COX-2 and is preferentially expressed in cerebral cortex and heart². The existence of a COX-3 was also proposed by Gilroy, *et al*²⁸, who showed that resolution of inflammation in the rat pleurisy model of inflammation was associated with increased expression of a COX-2-like protein. These authors hypothesized that this enzyme may produce PG that are involved in the resolution of inflammation. In contrast to the NSAID-inducible COX-2 in macrophages that produced PGE₂ in response to exogenous arachidonic acid³⁰, the COX-2-like form appearing during the resolution of inflammation as well as the 15d-PGJ₂-inducible COX-2-like form did not produce PGE₂ in response to exogenous arachidonic acid^{26,28}. Together, these findings suggest that additional COX-2-derived forms exist: they are likely distinct from the brain-specific and the NSAID-inducible COX-2-like form.

Prostaglandins exert diverse and complex modulatory roles in physiological and pathophysiological conditions such as cancer, inflammation, and arthritis. PGE₂ is known to play an important role in inflammation and arthritis, while emerging evidence indicates that another PG, 15d-PGJ₂, has antiinflammatory and antiarthritic properties. Since both PG are produced by a common COX enzyme, their relative rates of production should depend on the relative efficiencies with which they are converted by respective terminal synthases. Modulating the expression/activity of these

enzymes constitutes a therapeutic target that may provide further specificity. For instance, in contrast to COX inhibitors, mPGES inhibitors could block PGE₂ synthesis without interfering with the synthesis of other prostaglandins that may have antiinflammatory functions.

JOHANNE MARTEL-PELLETIER, PhD;
JEAN-PIERRE PELLETIER, MD;
HASSAN FAHMI, PhD.
Osteoarthritis Research Unit,
Centre Hospitalier de l'Université de Montréal,
Hôpital Notre-Dame,
1560 Sherbrooke Street East,
Montreal, Quebec, Canada H2L 4M1

Address reprint requests to Dr. Fahmi. E-mail: h.fahmi@umontreal.ca

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