

Overview of the Physiology of COX-1 and COX-2 and Selectivity of NSAIDs

Based on a presentation by Jan Åke Lindgren, MD, PhD

Presentation Summary

The discovery of two separate prostaglandin-producing cyclooxygenases in the late 1980s has revolutionized our view of how prostaglandin biosynthesis is regulated. This has also generated novel knowledge concerning the effects and side effects of nonsteroidal anti-inflammatory drugs (NSAIDs).

Prostaglandins are responsible for normal physiological functions and for modulating the inflammatory reaction. Whereas cyclooxygenase-1 (COX-1) maintains the beneficial, protective effects of prostaglandins, the cyclooxygenase-2 (COX-2) pathway leads to

increased inflammation. Therefore, drugs that can block COX-2 without disturbing the function of COX-1 may be ideal for alleviating pain and inflammation without adverse side effects. Further research, however, has indicated that COX-2 may also be involved in other functions, and that COX-1 activity may be related to inflammation under certain conditions.

Despite these questions, the new knowledge has opened possibilities for development of selective NSAIDs with less adverse side effects. However, more research is needed before determining the precise actions of these drugs.

New findings concerning the cyclooxygenase pathway have provided important information relevant for our understanding of the pharmacology of nonsteroidal anti-inflammatory drugs (NSAIDs). These drugs are believed to exert their action via inhibition of prostaglandin (PG) production, said Jan Åke Lindgren, MD, PhD, Associate Professor, Department of Medical Biochemistry and Biophysics, Karolinska Institute, in Stockholm.

Arachidonic acid, which is the main precursor of PGs, is not present as a free fatty acid in the cell, but is instead esterified to the phospholipids in the membranes. Activation of the cells stimulates phospholipase (PL)A₂, which liberates the fatty acid and allows arachidonic acid to be the substrate for cyclooxygenase. This enzyme catalyzes formation of the unstable endoperoxide prostaglandin

H₂, which is then further transformed into the various prostaglandins and other prostanoids (Figure 1).

“Prostaglandins are short-lived compounds in the body. For example, the half-life of PGE₂ is about 30 seconds.

“Prostaglandins are known to be involved in inflammation, but are also responsible for various ‘housekeeping functions,’ which protect normal physiological functioning.”

—Dr. Jan Åke Lindgren

This means that prostaglandins cannot function as classical hormones, but are instead local hormones acting at the place of formation. Prostaglandins are known to be involved in inflammation, but are also responsible for various ‘housekeeping functions,’ which protect

normal physiological functioning," Dr. Lindgren said.

Some of the most important of these protective PG functions occur in the gastrointestinal (GI) tract, the kidney, and

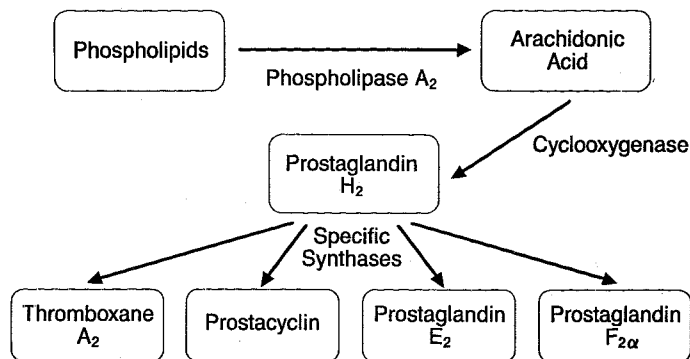
stacyclin) is that they are very potent vasodilators. This leads to potentiation of the action of other inflammatory mediators such as leukotrienes, histamine, bradykinin, and complement factors. The resulting increased vasopermeability leads to edema. In contrast, prostaglandins alone don't induce leakage by increasing vasopermeability," Dr. Lindgren said.

In addition, prostaglandins contribute to peripheral pain. While they do not produce pain by themselves, they potentiate the effects of other modulators through vasodilation and induction of hyperalgesia. Prostaglandins are also believed to be involved in the generation of fever. Thus, inflammatory cytokines, such as interleukin-1 (IL-1) induce production of PGE₂ in the hypothalamic, thermoregulatory center and this leads to increased body temperature.

Until recently, it was believed that administration of NSAIDs to alleviate inflammation, pain, and fever necessarily also had to cause suppression of the PG "housekeeping" functions, thereby generating unwanted drug side effects. However, the discovery of a second cyclooxygenase isoenzyme, cyclooxygenase-2 (COX-2), has changed this view. "Cyclooxygenase-1 (COX-1) is the enzyme involved in the formation of PGs for 'housekeeping' functions. This enzyme is commonly distributed in various cell types and is constitutively expressed. On the other hand, COX-2 is normally absent, but the expression of this enzyme can be induced by various inflammatory modulators. When present, COX-2 produces PGs responsible for modulating inflammation," said Dr. Lindgren. COX-2 expression has been demonstrated in macrophages, certain other leukocytes, fibroblasts, endothelial cells, and in the synovium of rheumatoid arthritis patients (Figure 2).

Several studies have indicated that COX-2 is the enzyme of importance in inflammation. For example, the expression of COX-2 has been demonstrated to increase in parallel to edema formation and PG production in experimen-

Figure 1. The Cyclooxygenase Pathway



the cardiovascular system. In the GI tract, PGE₂ and prostacyclin inhibit acid secretion, and stimulate bicarbonate and mucous secretion. There is also evidence that these compounds have a specific cytoprotective effect. Together these effects contribute to the maintenance of mucosal integrity.

In the kidney, PGs increase the blood flow, thereby elevating glomerular filtration, and participate in the regulation of tubular salt and water resorption. Prostacyclin and thromboxane A₂ are also important in hemostasis by maintaining the vascular tone and control of platelet function.

The Role of Prostaglandins in Inflammation

Under inflammatory conditions, huge amounts of PGs are formed and can be isolated from, for example, the synovial fluid of patients with rheumatoid arthritis. Administration of NSAIDs inhibits prostaglandin formation in inflammation.

"One important feature of the inflammatory prostaglandins (PGE₂ and pro-

tally induced inflammation, whereas COX-1 expression was constant throughout the inflammatory process. Also, COX-2 antisense oligonucleotides completely blocked endotoxin-induced PGE₂ formation in macrophages, even though these cells also contained COX-1, which was demonstrated by their ability to transform exogenously added arachidonic acid to PGs.

“Much evidence indicates that this happens because cytosolic phospholipase A₂ (cPLA₂), which is also induced during inflammation, liberates arachidonic acid and supplies this substrate to COX-2, but not to COX-1 (Figure 3). Cytosolic cPLA₂ and COX-2 have identical subcellular localization, the nuclear envelope, and the same inflammatory modulators stimulate the expression of both,” Dr. Lindgren said. Thus, the inflammatory cytokines IL-1 and tumor necrosis factor, as well as lipopolysaccharide, induce the expression of these enzymes, whereas anti-inflammatory cytokines, such as TGF-β, IL-10, IL-4, and IL-13, have been reported to inhibit the expression. Interestingly, glucocorticoids inhibit the expression of cPLA₂ and COX-2 (Figure 4).

In rheumatoid arthritis, for example, the expression of cPLA₂, COX-1, and COX-2 in the inflamed tissue has been demonstrated. However, it may thus be speculated that COX-1 is less involved in the formation of PGs during these conditions. Although COX-1 is present, it probably cannot be supplied with arachidonic acid, at least not from cPLA₂, according to Dr. Lindgren (Figure 5).

Other COX-1 and COX-2 Functions

In addition to its role in inflammation, COX-2 is apparently involved in other processes. The enzyme has been reported to be induced during ovulation, implantation, and labor induction and may therefore play a significant reproductive role. Interestingly, COX-2 may also be involved in the pathogenesis of colon cancer. “It has been known for a

Figure 2. Role of COX-1 and COX-2

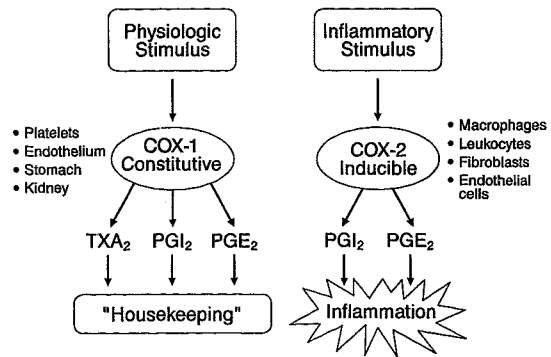


Figure 3. Cytosolic PLA₂ Provides Arachidonic Acid to COX-2, but not to COX-1

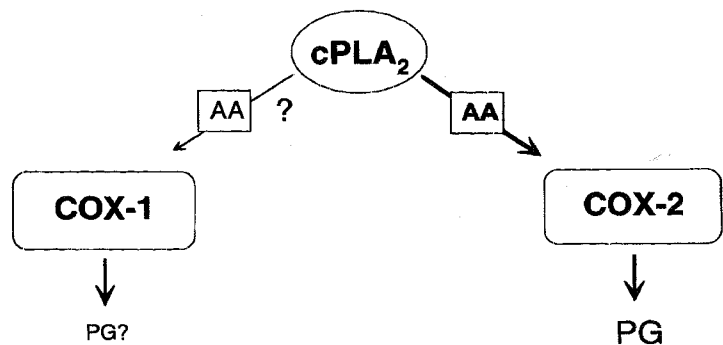
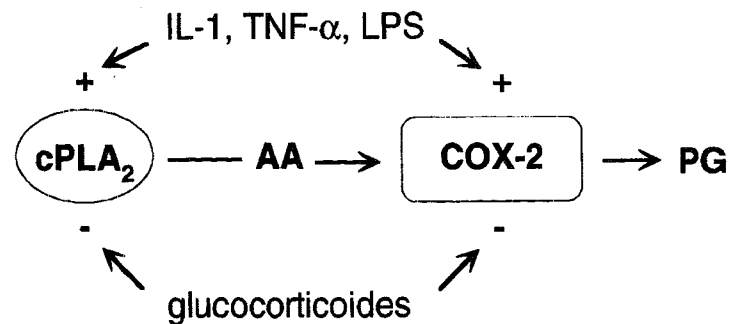


Figure 4. Coordinated Induction of cPLA₂ and COX-2 in Inflammation

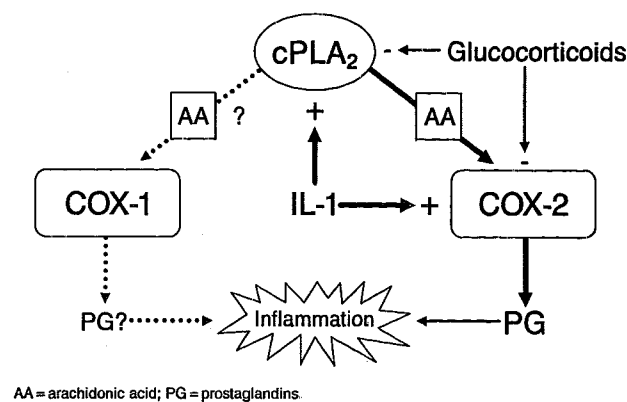


few years that NSAID treatment lowers the incidence of colon cancer and that these tumors produce increased prostaglandin levels,” Dr. Lindgren said. “And very recently it was found that neoplastic tissue from the colon expresses markedly elevated levels of COX-2 both at the messenger RNA and protein levels.”

PG formation has been described in activated mast cells and may very well occur under certain inflammatory conditions. Reports of sPLA₂ being active in synovial tissue from patients with rheumatoid arthritis support such a theory.

In experiments with knock-out mice, it has been demonstrated that COX-1 gene disruption impaired platelet function, as expected. Furthermore, animals lacking the COX-1 gene showed decreased inflammatory response to administration of exogenous arachidonic acid, demonstrating that this inflammatory model is mainly COX-1 dependent. “If arachidonic acid is added, and COX-1 is there, of course it will lead to generation of inflammatory prostaglandins,” Dr. Lindgren said. “Somewhat unexpectedly, the mice lacking the COX-1 gene did not develop stomach ulcerations, although gastric PGE₂ production was essentially absent. However, this may suggest that the absence of PG production in the stomach was compensated by the induction of another protective system (perhaps nitric oxide) in order to preserve mucosal integrity. In accordance to this theory, COX-1 gene disruption led to decreased gastric sensitivity to indomethacin.

Figure 5. Prostaglandin Production in Rheumatoid Arthritis



The function of COX-2 in colon cancer is not yet clear. It may be that prostaglandin production is not important but that cyclooxygenase activates certain carcinogens. However, Dr. Lindgren continued, it has been speculated that selective COX-2 inhibitors will be more suitable than conventional NSAIDs for colon cancer treatment because of their lack of gastric side effects.

Whether or not COX-1 can play a role in inflammation along with COX-2 is presently unknown. However, the involvement of COX-1 in inflammation has been speculated. “Just as cPLA₂ supplies COX-2 with arachidonic acid, it seems that COX-1 can be supplied with arachidonic acid by another phospholipase, the secretory phospholipase A₂ (sPLA₂),” Dr. Lindgren explained. Cell activation leads to stimulation of sPLA₂, which results in PG formation via the COX-1 pathway. This route of

During COX-2 gene disruption experiments, induction with lipopolysaccharide failed to induce PGE₂ production in peritoneal macrophages, suggesting that inflammation induced by cytokines and inflammatory modulators mainly involves COX-2. An unexpected result, however, was that the inflammatory response to carrageenan or bacterial invasion of the peritoneal cavity was normal. The reason for this is unknown, although the results may suggest a role for COX-1 in inflammation. In the COX-2 gene disruption model, female fertility was blocked, and there was also abnormal postnatal kidney development, indicating that COX-2 is involved in reproduction and developmental processes.

Selective COX-2 Inhibitors

Several compounds that selectively inhibit COX-2 formation, thereby

eliminating inflammatory PGs while preserving the PGs involved in necessary physiologic functions, have been developed and studied in the laboratory. Some of these drugs are now in clinical trials. In animal experiments they have been shown to be anti-inflammatory, analgesic, and antipyretic, without being ulcerogenic.

For example, an investigational COX-2 inhibitor, NS-398, was found to be equally effective as indomethacin for alleviating inflammation. However, whereas indomethacin and other conventional NSAIDs inhibited stomach PG synthesis and induced gastric ulcers, NS-398 did not have these effects. Similar findings have been reported also for other COX-2 inhibitors. "This indicates again that inflammation is mainly mediated by COX-2, while COX-1 is the important enzyme for the protection of the gastric mucosa," according to Dr. Lindgren.

Most conventional NSAIDs are COX-1 inhibitors with much higher doses needed to inhibit COX-2. The active metabolite of nabumetone, 6-MNA, appears to be an exception. Different studies have produced somewhat variable results, indicating that 6-MNA has anywhere from similar effects on COX-1 and COX-2, to up to seven times higher efficacy in inhibiting COX-2. However, variable results have also been reported for other NSAIDs (Tables 1 and 2). Despite this confusion, it is still possible to see a pattern where some NSAIDs, including indomethacin, sulindac, and piroxicam have generally been demonstrated to possess high selectivity for COX-1 inhibition and are associated with a high degree of GI toxicity. Ibuprofen, naproxen, and diclofenac produce intermediate COX-2 to COX-1 ID₅₀ ratios and are also reported to provoke less gastric toxicity.

"Although 6-MNA behaves clinically as a selective COX-2 inhibitor, this has not been fully demonstrated in either *in vitro* or *in vivo* experiments," Dr. Lindgren said. "One reason could be dif-

Table 1. Inhibitory Effects of 6-MNA on COX-1 and COX-2

Reference	System	IC ₅₀ (μM)		COX-2
		COX-1	COX-2	COX-1
Meade et al, 1993	Membranes, cells transf. with murine enzyme	240	35	0.14
Barnett et al, 1994	Human recombinant enzymes	70	20	0.29
Laneville et al, 1994	Membranes, cells transf. with human enzyme	64	94	1.5
Patrignani et al, 1994	Human whole blood	278	187	0.67

Table 2. Human Recombinant COX-1 and COX-2 Inhibition by NSAIDs

NSAID	IC ₅₀ , μM		Ratio
	COX-1	COX-2	
Indomethacin	1.7	25	14.7
Flurbiprofen	0.04	0.51	12.8
Ketoprofen	0.50	2.3	4.6
Naproxen	0.60	2.0	3.3
Diclofenac	0.90	1.5	1.7
Ibuprofen	2.6	1.5	0.58
6-MNA	70	20	0.29

From Barnett et al, *Biochem Biophys Acta* 1994;1209:130.

iculties in defining proper test systems." There may also be other explanations for the beneficial clinical effects of nabumetone, such as differences in tissue distribution and cellular uptake of 6-MNA.

"In the future, selective COX-2 inhibitors will hopefully be proven to be anti-inflammatory without inducing adverse side effects," Dr. Lindgren concluded.