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Review

Prostaglandins and Inflammation: the Cyclooxygenase Controversy

OLIVIER MORTEAU*

Department of Pulmonary Medicine, Children's Hospital, Harvard Medical School, 300 Longwood Ave, Boston, MA 02115, USA

Abstract. Prostaglandins (PGs) are arachidonic acid metabolites produced by the action of the enzyme cyclooxygenase (COX). Although PGs are important mediators of inflammation in various diseases, they also are key factors in the physiological regulation of gastrointestinal and renal homeostasis. The finding that two distinct COX isoforms are responsible for PG synthesis has provided basis to the opposite actions of PGs in inflammation and homeostasis regulation. COX-1-derived PGs are thought to mediate cytoprotective actions on the gastrointestinal mucosa, whereas COX-2-derived PGs are assumed to display pro-inflammatory properties. This dichotomy has led to the development of selective inhibitors of COX-2 activity which are safer for the gastrointestinal mucosa than the classic inhibitors of both COX isoforms. However, some COX-2 anti-inflammatory properties have been recently demonstrated in several experimental models of inflammation. These studies have raised some concern about the potential adverse effects of COX-2 selective inhibitors. In addition, there is evidence that COX-1 displays pro-inflammatory properties, depending on the organ and on the stage of the inflammatory response. This review will focus on the roles of COX-1 and COX-2 in inflammation, based on studies involving pharmacologic COX inhibitors as well as COX knock-out mice, with a particular emphasis on the gastrointestinal tract.

Key words: prostaglandins; cyclooxygenase; nonsteroidal anti-inflammatory drugs (NSAIDs); inflammation; colitis.

Arachidonic acid (AA), produced by the action of phospholipases on cell membrane phospholipids, is the substrate for the homodimeric enzyme cyclooxygenase (COX) or prostaglandin H (PGH) synthase (Fig. 1). COX catalyzes the synthesis of prostaglandins (PGs) through a two-step process. A di-oxygenase activity first cyclizes and oxygenates AA into PGG_2. Then, a peroxidase activity catalyzes the reduction of PGG_2 into PGH_2. PGH_2 is the precursor of PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and thromboxane A_2 (TXA_2), which are

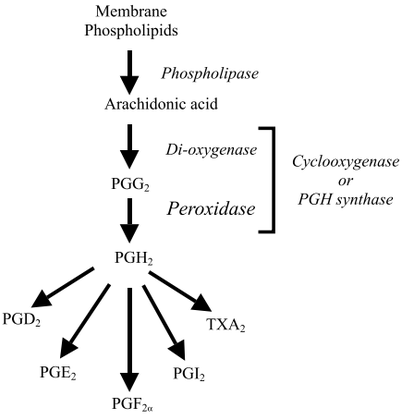

Fig. 1. Pathway of protaglandin biosynthesis

* Correspondence to: Dr Olivier Morteau, Children's Hospital Medical Center, Enders 136, 61 Binney St., Boston, MA 02115, USA, tel.: +1 617 355 69 38, fax: +1 617 738 17 52, e-mail: morteau_0@a1.tch.harvard.edu

synthesized via cell-specific synthases. PGs are important mediators of inflammation. In addition, they play a critical role in the protection of the gastrointestinal mucosa and in the regulation of renal homeostasis.

Prostaglandins in Inflammation and Homeostasis Regulation

Prostaglandins mediate various manifestations of the inflammatory response, including fever, hyperalgesia, increase in vascular permeability and edema. In the intestine, PGs influence motility and secretion and they stimulate diarrhea by promoting chloride secretion and blocking sodium absorption in epithelial cells[61]. PGs are involved in a number of inflammatory conditions and diseases, such as arthritis[8], skin inflammation where PGs potentiate the inflammatory actions of platelet-activating factor (PAF)[20] and asthma where PGD_2 mediate bronchoconstriction[24]. Classic nonsteroidal anti-inflammatory drugs (NSAIDs) block PG synthesis by inhibiting both COX-1 and COX-2 enzymatic activities. They display anti-inflammatory, analgesic, and antipyretic properties[1]. NSAIDs are widely prescribed for the treatment of many conditions, including rheumatoid arthritis, osteoarthritis, gouty arthritis, the joint and muscle discomfort associated with systemic lupus erythematosus, and other musculoskeletal disorders[56].

The role of PGs in the gastrointestinal tract is more confusing. Dramatic increases in mucosal PG synthesis correlate with disease activity of the human inflammatory bowel disease (IBD) and of experimental colitis[2, 55, 67]. However, PGs also exert a protective role against gastrointestinal injury[26], possibly by down-regulating the expression of proinflammatory cytokines[32, 42]. Indeed, experimental colitis can be attenuated by pretreatment with exogenous PGs[2, 13] and PGE_2 plays a major role in the regeneration of the epithelial crypts following intestinal damage induced in mice by radiation[7] and by the chemical dextran sodium sulfate (DSS)[72]. In genetically susceptible rats, inhibition of PG synthesis by the NSAID indomethacin induces acute and chronic enterocolitis[77]. Similarly, active or passive immunization with PGE_2, PGE_1 and prostacyclin (PGI_2) induces gastrointestinal ulcers in rabbits[53, 58].

In addition, PGs are important physiological modulators of vascular tone and salt and water homeostasis in the normal mammalian kidney[22].

Adverse Effects of NSAIDs in the Gastrointestinal Tract

More evidence for a protective role of PGs in the gastrointestinal mucosa is supported by the clinical side-effects of NSAIDs on the digestive tract[4, 30]. The most serious side-effects of NSAIDs involve gastroduodenal perforations and massive hemorrhage[5, 56]. According to BJARNASON and MACPHERSON[5], 70% of patients receiving NSAIDs in the long term suffer from small intestinal inflammation, which leads to a loss in blood and proteins. NSAIDs have been held responsible for 12 000 ulcer bleeding episodes and 1200 deaths per annum in the United Kingdom[23]. Other side-effects of NSAIDs include suppression of hemostasis through inhibition of platelet aggregation, adverse effects in patients with heart failure and cirrhosis and those with certain renal diseases, as well as complicating therapies involving diuretics or β-adrenoceptor blockade[56].

The adverse effects of classic NSAIDs are partly independent of COX inhibition. For example, NSAIDs may intercalate into the lipid bilayer of the plasma membrane and thereby disrupt protein-protein and protein-lipid interactions critical to cell responses, such as calcium translocations and membrane phospholipid turnover[1]. Some NSAIDs may exert direct inhibitory actions at a regulatory G protein within the plasma membrane[1]. NSAIDs may also inhibit mitochondrial oxidative phosphorylation[23]. In addition to the inhibition of COX activity, some NSAIDs also inhibit the lipoxygenase pathway that leads to the formation of leukotrienes[6]. In addition, it has been suggested that the analgesic effect of NSAIDs is mediated at central level through the action of endogenous opioid peptides or the blockade of the release of serotonin[6].

The COX-1/COX-2 Dichotomy: a New Concept for Safer Drugs

Two 70 kDa isoforms of COX (COX-1 and COX-2) encoded by two distinct genes have been identified[33, 34, 52, 75, 76, 78]. Opposite roles have been attributed to these two isoforms, based on major differences in the regulation of their expression. COX-1, whose expression is constitutive in most tissues and unaffected by glucocorticoids, is thought to maintain physiological homeostasis in the gastrointestinal tract. By contrast, COX-2 is only constitutive in parts of the kidney and of the brain, in fetal membranes and in the uterus during pregnancy, and in endothelial cells, smooth muscle cells, osteoblasts, mesangial cells and rheumatoid synoviocytes. In all other tissues and cells, COX-2 is inducible by a variety of mitogens, tumor promoters and proinflammatory stimuli, including bacterial factors and cytokines (for review, see[25]). In human and murine fibroblasts, COX-2 expression can be induced by TPA, serum, interleukin

-1β (IL-1β), tumor necrosis factor α (TNF-α), epidermal growth factor (EGF) and platelet-derived growth factor. In the human and murine macrophage, COX-2 is inducible by lipopolysaccharides, interferon γ (IFN-γ) and IL-1-β. In gastrointestinal epithelial cells, COX-2 expression can be induced by EGF and transforming growth factor β. The induction of COX-2 expression by TNF-α in colonic epithelial cells is mediated by the activation of the nuclear factor NF-κB[27], a major transcription factor for cytokines. COX-2 over-expression in rat epithelial cell lines inhibits the expression of cytoprotective heat-shock proteins[12]. In addition, COX-2 mRNA and protein expressions are inhibited at both transcriptional and post-transcriptional levels by glucocorticoids such as cortisol and dexamethasone[35, 43, 57].

Based on these differences in expression, the concept of "a pro-inflammatory COX-2" versus a "protective COX-1" rapidly emerged[9, 64, 65]. This dichotomy has provided an explanation to the ulcerogenic actions of the classical NSAIDs, which inhibit both COX-1 and COX-2 isoforms[45]. The anti-inflammatory properties of NSAIDs were explained by the inhibition of COX-2--derived pro-inflammatory PGs, while the side-effects of these drugs on the gastrointestinal mucosa were explained by the inhibition of a constitutive pool of COX--1-derived protective PGs (Fig. 2). This theory implied the existence of two distinct sets of PGs with opposite actions, depending on their cellular origin and on the nature of the target organ. As a direct consequence, selective inhibitors of COX-2 activity were developed, in the hope that they would display similar anti-inflammatory properties to classical NSAIDs while being safer for the gastrointestinal mucosa. Selective COX-2 inhibitors have been reported to reduce air pouch and foot pad experimental inflammation in animal models without causing gastrointestinal injury, in contrast to classical NSAIDs[44, 66]. Several recent clinical trials have confirmed the safety of selective COX-2 inhibitors. The selective COX-2 inhibitors rofecoxib and celecoxib caused significantly less gastroduodenal ulceration than classic NSAIDs in patients with osteoarthritis or rheumatoid arthritis while displaying equivalent anti-inflammatory and analgesic efficacy[18, 38, 41, 73].

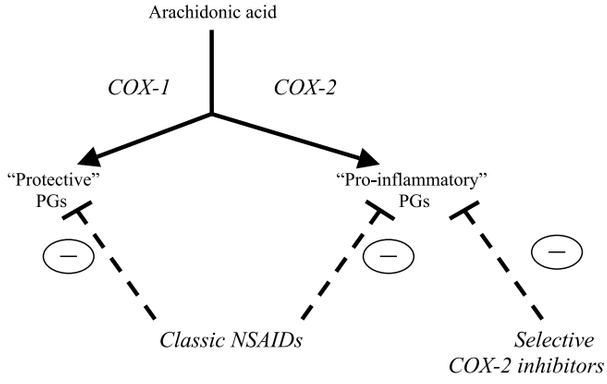

Fig. 2. Dichotomy between "protective" COX-1 and "pro-inflammatory COX-2" and inhibitory actions of classic NSAIDs and of slective COX-2 inhibitors

COX Knock-Out Mice: a Complement to Pharmacologic Studies

In complement to the selective COX-2 inhibitors, mice genetically deficient in COX-1[40] and in COX-2[47] have been important tools for investigating the relative contribution of both COX isoforms in inflammation and in physiological processes such as pregnancy, kidney development and carcinogenesis.

In a model of ear inflammation induced by AA, COX-1$^{-/-}$ but not COX-2$^{-/-}$, mice exhibited a 70% reduction in edema when compared with wild-type mice[40], suggesting that COX-1 may mediate the initial inflammatory response to an agent that causes rapid AA release[39]. COX-deficient and wild-type mice were equally affected in a model of ear inflammation induced by the tumor promoter TPA[40, 47]. Furthermore, COX-2$^{-/-}$ and wild-type mice exhibited similar responses in a model of paw edema induced by carrageenan[10, 74]. However, indomethacin, but not a selective COX-2 inhibitor, significantly inhibited carrageenan-induced edema in both wild-type and COX-2$^{-/-}$ mice[74], indicating that significant inhibition of PG synthesis occurs only at NSAID doses that also inhibit COX-1[39]. In the carrageenan-induced air pouch model, both COX-2 deficiency and pharmacologic COX-2 inhibition[14, 15] were shown to be the main pathway for PG production during the early stages of inflammation, with COX-1 contributing about 25% of the PGs produced[39]. Recently, allergic lung responses were reported to be increased in both COX-1- and COX-2-deficient mice[16]. In this study, COX-1 was shown to be the major source of PGE_2 in the normal mouse lung, both COX-1 and COX-2 products limited allergic lung inflammation and IgE secretion and promoted normal lung function, and airway inflammation could be dissociated from airway hyperresponsiveness in COX-2$^{-/-}$ mice[16]. Taken together, these observations suggest that both COX-1 and COX-2 contribute to the inflammatory response and that the extent to which each isoform contributes PGs may depend on the inflammatory stimuli, the time after insult, and the relative levels of each isoform in the target tissue[40].

COX-2 in Gastrointestinal Inflammation: a Protective Role

The first pieces of evidence supporting a beneficial role for COX-2 in gastrointestinal inflammation were brought by experimental studies in rodents. Using a model of colitis induced in rats by rectal administration of 2,4,6-trinitrobenzene sulfonic acid (TNBs) in 50% ethanol, REUTER et al.[59] demonstrated that the severity of the disease is exacerbated by the administration of the selective COX-2 inhibitor etodolac. Following this observation, MIZUNO et al.[46] showed that selective inhibition of COX-2 by NS-398 delays the healing of gastric ulcers induced by acetic acid in mice. This study was in agreement with two other reports that selective COX-2 inhibitors can delay gastric ulcer healing in rats[69] and increase the severity of stress-induced gastric ulcers in rats[50].

In complement to these observations, we have investigated the impact of either the COX-1[39] or COX-2[47] genetic deficiency on the severity of murine colitis induced by oral administration of DSS[49]. Naive, untreated COX-1$^{-/-}$ and COX-2$^{-/-}$ mice did not display any feature of spontaneous intestinal inflammation, even-though basal gastrointestinal tissue levels of PGE_2 were barely detectable in the COX-1$^{-/-}$ mice when compared with naive wild-type and COX-2$^{-/-}$ mice[49]. This confirmed that COX-1 is the constitutive source of PGs in the normal gastrointestinal mucosa and that neither COX-1 nor COX-2 activity alone is required to maintain mucosal homeostasis in the absence of injurious stimuli. When treated with DSS, wild-type, COX-1$^{-/-}$ and COX-2$^{-/-}$ mice all exhibited colitis characterized by progressive body weight loss and bloody diarrhea. COX-1$^{-/-}$ and COX-2$^{-/-}$ were significantly more affected than wild-type mice, with higher clinical scores and decreased survival, and the disease was more severe in the COX-2$^{-/-}$ than in the COX-1$^{-/-}$ mice. COX-2$^{-/-}$ mice exhibited significant blood changes, macroscopic alterations of the colon and spleen and a significant increase in colonic epithelial damage. In addition, the severity of DSS-induced colitis was exacerbated in COX-1$^{-/-}$ mice treated with a selective COX-2 inhibitor[49]. It was argued that intestinal PG production might be abnormally regulated in COX-1 and COX-2 knock-outs and that these mice might display compensatory metabolic pathways. This was described in immortalized, nontransformed lung fibroblasts from COX-1$^{-/-}$ and COX-2$^{-/-}$ mice, where the remaining functional COX isoform is overexpressed in response to IL-1, leading to a relative increase in PGE_2 production[31]. These cells also express cytosolic phospholipase A_2 in both unstimulated and IL-1-stimulated conditions[31]. However, colonic PGE_2 production in our colitis model was dramatically increased in both wild-type and COX-1$^{-/-}$ mice, but not in COX-2 knock-outs[49]. This implied that COX-2 is the major source of PGs during colitis and that there is no compensatory metabolic pathway in the colons of these knock-out mice. Taken together, our observations demonstrated that both COX-1 and COX-2 isoforms play a protective role during intestinal inflammation, although neither isoform alone is required to maintain basal mucosal homeostasis in normal conditions.

The mechanisms of action of COX-1 and COX-2 in intestinal injury are still under investigation. PGE_2 inhibits the secretion of the proinflammatory cytokines IL-1 and TNF-α by stimulated human[32] and murine[36] monocytes, providing one mechanism of mucosal protection during inflammation. This hypothesis is supported by our observation that the inflamed colons of DSS-treated COX-2$^{-/-}$ mice produce significantly more IL-1β and less PGE_2 than the inflamed colons of DSS-treated wild-type mice[49]. The recent observation that both selective COX-2 inhibitors and classic NSAIDs can inhibit angiogenesis through direct effects on endothelial cells indicates that COX-2 is important for the regulation of angiogenesis and provides a possible mechanism for the positive role of COX-2 in gastrointestinal protection[28].

COX-2 in Oral Tolerance: a Modulator of the Intestinal Immune Response

Besides its protective role in experimental gastritis and colitis, COX-2 has recently been reported to mediate oral immunologic tolerance to a dietary antigen in T cell receptor (TCR) mutant mice[51]. Oral tolerance, a state of immunologic hyporesponsiveness to dietary antigens, is the protective mechanism by which the immune response of the intestinal epithelium to dietary antigens is effectively modulated through activation of specific subsets of T lymphocytes. In the study of NEWBERRY et al.[51], oral administration of a specific dietary antigen to TCR transgenic mice resulted in no intestinal pathology, while coadministration of the antigen and of selective COX-2 inhibitors induced an intestinal inflammation characterized by villus erosion, crypt expansion and proliferation of lamina propria mononuclear cells (LPMNCs). Furthermore, LPMNCs from COX-2$^{-/-}$ mice produced less than 1% of the PGE_2 produced by wild-type or COX-1$^{-/-}$ LPMNCs, which demonstrates that the high levels of PGE_2 produced by

LPMNCs during oral tolerance depend on the function of COX-2 but not COX-1[51]. This study suggests that COX-2 may promote intestinal mucosal protection by suppressing pathogenic cellular immune responses to luminal bacterial antigens which perpetuate intestinal inflammation[48]. This provides a complementary mechanism to explain the protective action of COX-2 during intestinal inflammation.

Selective COX-2 Inhibitors: More Concern... More Hope

Taken together, the data on experimental colitis raise the concern that selective COX-2 inhibitors may be harmful in patients with a history of gastric or intestinal inflammation. A typical example would be patients with IBD treated for extra-intestinal manifestations of the disease, like arthritis. Raising a more general concern, it has recently been suggested that COX-2 plays both a pro-inflammatory and an anti-inflammatory role, depending on the stage of the inflammatory response[17]. In a model of carrageenan-induced pleurisy in rats, both the selective COX-2 inhibitor NS-398 and the dual COX-1/COX-2 inhibitor indomethacin inhibited inflammation at 2 h and exacerbated inflammation at 48 h. It was concluded that COX-2 may be pro-inflammatory in the polymorphonuclear leukocyte-dominated, early phase of a carrageenan-induced pleurisy, and may regulate resolution in the later, migrating mononuclear cell-dominated phase, by generating an alternate set of PGs such as those of the cyclopentenone family (PGD_2 and $15deoxy\Delta^{12-14}PGJ_2$)[17].

The kidney may be another source of concern related to the use of selective COX-2 inhibitors. In contrast to most organs, the normal kidney constitutively expresses both COX isoforms and their respective roles in renal physiology are still under investigation. COX-2 expression is enhanced in the early phase of mesangioproliferative glomerulonephritis in rats, suggesting that COX-2 may play a major role in the mediation of inflammatory cell recruitment in this disease and that selective inhibition of COX-2 might reduce nephrotoxic side effects in renal inflammation[63]. However, studies indicate that COX-2 may also play an important role in the regulation of salt, volume, and blood pressure homeostasis, through regulation of renin release[21]. Furthermore, observations in the $COX\text{-}2^{-/-}$ mice suggest that COX-2 is a critical factor in the postnatal development of the kidney[10, 47]. As stated by SCHNEIDER and STAHL[63], the exact physiological role of COX-2 in human kidneys is still unclear, and there is no current evidence justifying the use of COX-2 inhibitors in renal disease.

An adverse response to COX-2 inhibition has also been recently demonstrated in the brain. A selective COX-2 inhibitor has been shown to aggravate experimental seizure and neuronal cell death in the hippocampus of mice treated with kainic acid[3]. Although this observation is isolated, it warns us of the potential side effects of COX-2 inhibitors on the central nervous system.

In contrast to these studies, it has been suggested that selective COX-2 inhibitors may be useful as anti-tumoral drugs[19]. COX-2 is thought to play a role in colorectal tumorigenesis[11, 29, 37, 60, 68, 73], and both genetic and pharmacologic inhibition of COX-2 suppress intestinal polyposis in $Apc^{\Delta 716}$ deficient mice, a model of familial adenomatous polyposis[54]. However, COX null fibroblasts derived from embryos deficient in both COX-1 and COX-2 still undergo neoplastic transformation and NSAIDs exerts anti-tumorigenic properties in these cells, which suggests that some of the anti-proliferative and anti-neoplastic properties of NSAIDs are independent of the inhibition of either COX-1 or COX-2, and that transformation is independent of the status of COX expression[79]. Very recently, it was shown that both selective COX-2 inhibitors and classic NSAIDs can inhibit angiogenesis through direct effects on endothelial cells involving both PG-dependent and PG-independent components[28]. This suggests a possible application of COX-2 inhibitors in cancer therapy[62]. Although more informations are needed on the role of COX-2 in cancer, selective COX-2 inhibitors are expected to bring promising chemotherapeutic effects[71].

In summary, combined observations based on the effects of classic NSAIDs, of selective COX-2 inhibitors and of the genetic absence of either COX-1 or COX-2 have been very helpful in investigating the relative roles of PGs in inflammation. Although COX-2 is the major source of proinflammatory PGs during inflammation in most organs, there is evidence that COX-1 is also involved, depending on the nature of the inflammatory stimuli and on the organ where the inflammation takes place. COX-2 is protective during gastrointestinal inflammation and may display anti-inflammatory actions in other organs, depending on the stage of the inflammatory response. Although selective COX-2 inhibitors are powerful anti-inflammatory drugs and may become anticancerous agents, their potential side effects on the inflamed gastrointestinal mucosa, on the kidney and on the brain should be further investigated.

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