Aspirin and the other NSAIDs have popularized the notion of inhibiting prostaglandins as a common anti-inflammatory strategy based on the erroneous premise that all eicosanoids are, within the context of inflammation, generally detrimental. However, our fascination with aspirin and the emergence of COX-2 has shown a more affable side to lipid mediators based on our increasing interest in the endogenous control of acute inflammation and in factors that mediate its resolution. Epi-lipoxins, for instance, are produced from aspirin’s acetylation of COX-2 and together with Resolvins and COX-2–derived prostaglandins of the D2 and J2 series represent an increasingly important family of immuno-regulatory lipid mediators with strong implications for disease control and drug discovery.
COX Metabolism and NSAIDs

Although AA may also be metabolized by cytochrome p450 systems, the focus of this review will be on the activities of COX and, to a lesser extent, LOX. There are three isoforms of COX, also known as prostaglandin G/H synthase. The first, COX-1, is constitutively expressed in most tissues and synthesizes prostaglandins (PGs) at low levels, and has thus been presumed to function primarily in the maintenance of physiological functions (5). COX-2, on the other hand, is highly inducible in response to pro-inflammatory stimuli, cytokines, and mitogens, and it has thus been generally conceptualized to function in the inflammatory release of PGs (6–10). Since the initial discovery of COX-2, however, three generalizations have been challenged. Phorbol esters, for example, support the differentiation of the TIB-73 human monocytic leukemia cell line, in part by inducing COX-1 (7, 11), and stem cell factor and dexamethasone induce COX-1 expression in mouse bone marrow–derived mast cells (12). Furthermore, COX-2 is expressed constitutively in discrete populations of neurons throughout the forebrain, as well as in the cortex and hippocampus; its expression is similarly constitutive in the macula densa of the juxtaglomerular apparatus and adjacent epithelial cells of the cortical thick ascending limb of the kidney (8, 7). In endothelial cells, however, its expression is somewhat controversial; some workers maintain that COX-2 is expressed in the endothelium under resting conditions in response to shear stress, for instance, or under circumstances of focal inflammation (e.g., atherosclerosis), whereas others detect no COX-2 expression in the endothelial cells from various vascular trees (9, 13, 14). Finally, COX-3, a splice variant of COX-1, is inhibited by acetaminophen and is thus thought to mediate the antipyretic and analgesic effects of this drug (15); however, the protein expression and functional relevance of acetaminophen-sensitive COX-3 in humans has been questioned (16).

COX converts AA, which is released from the plasma membrane via the action of phospholipase A2, into PGH2, and then into PGE2, Figure 1). The latter, in turn, acts as a substrate for a series of downstream synthases responsible for the generation of PGs, thromboxane A2, and prostacyclin (collectively called prostanoids) (Figure 1). Both COX-1 and -2 share similar structural properties, including a hydrophobic tunnel that allows AA access to the respective active sites. In COX-1, this hydrophobic tunnel has a side pocket, and the enzyme therefore manifests broader substrate recognition than does COX-1. This difference in substrate specificity will be important later when we discuss the ability of COX-2 to metabolize eicosapentaenoic acid and docosahexaenoic acid, either in the presence or absence of aspirin, to produce novel endogenous anti-inflammatory and pro-resolution lipid mediators. This structural distinction of the active site of COX-2 has lent itself to the discovery of compounds (i.e., the coxibs, e.g., rofecoxib (Vioxx, Merck) that selectively inhibit COX-2 (17) and were thus developed and marketed as anti-inflammatory drugs. The coxibs, moreover, were reputed, by virtue of their limited gastric toxicity (18, 19), to have an advantage over traditional NSAIDs; it was initially believed that adverse gastric effects typically stem from the inhibition of COX-1. It is now generally believed that both COX-1 and COX-2 need to be inhibited simultaneously in order to elicit gastric ulceration, inasmuch as selective inhibition by coxibs only reduced ulceration by 50% compared to NSAIDs (20). In addition, the reported expression of COX-2 in the vascular endothelium, along with its roles in resolving acute inflammation and in healing gastric ulcers, has impinged the use of COX-1 inhibitors for treating chronic inflammation. Indeed, the association of adverse cardiovascular events associated with Vioxx consumption for chronic rheumatic pain has led to the voluntary withdrawal of Vioxx from the market. In the next few paragraphs, we will recount important evidence from...
COX-2: In Inflammation and Resolution

The last ten years that shows COX2 to be a versatile enzyme in the inflammatory response. This single enzyme possesses the ability, under certain conditions, to drive inflammation, and remarkably, the ability to resolve inflammation under other conditions.

**NSAIDs and COX-2 Inhibitors**

The inhibition of PG synthesis accounts, at least in part, for the anti-inflammatory properties of the NSAIDs that are widely used for the treatment of inflammatory joint diseases. The use of traditional NSAIDs, however, is also associated with renal and gastrointestinal toxicity arising from the inhibition of COX-1 (20). Intriguingly, these COX-2 inhibitors showed little or no renal toxicity (21). In terms of basic research, the development of COX-deficient mice indicate that both COX-1 and COX-2 can contribute to the inflammatory response.

**COX-1 and COX-2, the “constitutive” COX-1 and inducible COX-2” isomers.**

COX-1 is expressed in a large number of tissues, including the brain, uterus, and vascular endothelium, and is responsible for the production of prostanoids that are involved in normal physiological processes, such as the maintenance of blood flow, the regulation of platelet aggregation, and the modulation of uterine contractility. COX-2, on the other hand, is induced in response to inflammatory stimuli and is responsible for the production of prostanoids that are involved in the resolution of inflammation and the repair of tissue damage.

**Inhibition of PG synthesis accounts, at least in part, for the anti-inflammatory properties of the NSAIDs that are widely used for the treatment of inflammatory joint diseases.**

**Figure 1.** The prostaglandin pathway. Synthesis of prostaglandin by the COX enzymes following the catalytic release of arachidonic acid from membrane phospholipids by calcium-dependent phospholipase A2. PGH2 is metabolized as indicated by specific synthases. Alternatively, arachidonic acid can be metabolized by lipoygenase or cytochrome P450 activities to leukotrienes, lipoxins, and hydroxy/epoxyeicosatrienoic fatty acids. Reproduced from (B7).

**Prostanoi**d | Predominant location | Function
---|---|---
**PGE**2 | Vascular endothelium and subendothelium | Inhibition of platelet aggregation and adhesion; broncho- and vasodilation; cholestatic effect on arterial cells; vascular leakage
**PGD**2 | Most cells; PGD synthase also expressed in brain | Bronchospasm and allergic asthma; inhibition of platelet aggregators; sleep
**PGF**2α | Renal medulla, gastric mucosa, platelets, microvascular endothelium | Inhibition of sodium reabsorption; broncho- and vasodilation; platelet contraction; lymphocyte function; presynaptic adrenergic modulation
**PGI**2 | Brain, venules | Bronchial and uterine contraction; urine vasoconstriction; partition

**Table 1.** A closer analysis reveals that COX2 inhibitors used in some of the early reports were administered at doses in excess of that required to inhibit COX2. For instance, in one camagostine air pouch study, the concentration of the highly selective COX2 inhibitor, SC-58125, required to inhibit ear swelling was a 100-fold greater than that necessary to inhibit ear exudate PG levels (27). Significantly, this compound is 100-fold more potent as an inhibitor of COX2 than of COX-1 in vitro; it is therefore possible that the SC-58125 doses used in this model inhibited COX-1 as well as COX-2. In addition to such pharmacological studies, the involvement of COX-2 in acute inflammatory models was similarly indicated in studies using carrageenin mice. AA induces edema in wildtype mice. AA induces edema in wildtype mice far more efficiently than in COX-1-deficient mice (28), suggesting a role for COX-1 and not COX-2 in this model of inflammation. Additionally, COX-1–deficient and wild-type mice were found to have similar model responses in terms of ear swelling (induced by trinitrobenzene sulfonic acid) and paw swelling (in response to carrageenin) (29). Because mice devoid of COX-2 still express COX-1, it is conceivable that this constitutive isoform synthesizes sufficient bioactive PGs for inflammation to proceed. Collectively, these studies in COX-1-deficient mice indicate that both COX-1 and COX-2 can contribute to the inflammatory response.

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Indeed, subsequent studies during the late 1990s began to suggest that in some tissues, most notably within the gastrointestinal system, COX-2 may be protective (31–33). In experimental models of colitis, for instance, the majority of PGs in the colonic mucosa are synthesized by COX-2 (34). Treatment with a selective COX-2 inhibitor (L-745,337) at doses that do not inhibit COX-1 depresses mucosal PG synthesis and causes a marked exacerbation of colonic damage (35). Continuous treatment for up to one week results in perforation of the bowel wall and death. It was also shown that COX-2 mRNA and protein are strongly induced in murine models of gastrointestinal ulceration, concurrent with increased mucosal PG synthesis (36, 37). Treatment of the mice with the selective COX-2 inhibitor NS-398 reduces mucosal PG synthesis and significantly inhibits ulcer healing (38). These results have been confirmed by others who also showed marked inhibition of gastric ulcer healing in rats by the COX-2 inhibitor L-745,337 at doses that selectively inhibit this inducible isozyme (39). These studies thus question whether gastroprotective PGs are synthesized solely by COX-1 and suggest that COX-2 plays a significant role in protecting the gastrointestinal tract from injury. As mentioned earlier, it now seems that inhibition of both COX-1 and COX-2 in healthy gastrointestinal tissues is required to elicit ulcers.

**Pro-resolution Properties of COX-2**

The findings with NSAIDs and COX-deficient mice indicate that both COX-1 and COX-2 contribute to PG production at the site of inflammation and also that COX-2-derived PGs have roles in the resolution and healing phase as well as in the early stages of the inflammatory response. In the seminal paper on the phenotype of COX-2 knockouts, the mice developed supplicative peritonitis with signs of inflammation that failed to resolve, whereas mice overexpressing the enzyme display few signs of inflammation (40). Treatment with a selective COX-2 inhibitor (L-745,337) at doses that selectively inhibit this inducible isozyme (39, 40) and continuous treatment for up to one week results in perforation of the bowel wall and death. This supports and complements the original findings with COX-2 in resolution of pleuritis as discussed above. Whether the absence of hPGD2S predisposes to chronic inflammation or autoimmunity has yet to be determined. Nonetheless, the clear lack of inflamma-

**Figure 2. Actions of PGD2 and 15-deoxy-12,14-PGD2 (PGD15) on T-lymphocyte functioning in this model appears to be mediated by 15d-PGJ2, and its inhibition of NF-κB DNA binding (Figure 2).**

**Contrary to the emerging literature, however, there was little apparent role for PGD2, acting through either of its receptors [i.e., the D prostaglandin (PGD) receptor and the chemokine receptor-homologue molecule expressed on Th2 cells (CD127/CCR2)] in controlling lymphocyte function. These findings suggest an important role for both COX-2 and hPGD2S as checkpoint controllers in the progression from acute to resolving inflammation (Figure 2).** In ongoing work, we have found an equally important role for both COX-2 and hPGD2S in the regulation of the onset phase of innate peritonitis as well as in its resolution through the regulation of macrophage clearance to the draining lymphatics and regulatory lymphocyte function (S Rajakumar and DW Gilroy).

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Aspirin and COX-2 in Inflammation and Resolution

As with the COX arm of the AA pathway, the LOX pathway is not wholly pro-inflammatory, with certain PG metabolites possessing either pro- or anti-inflammatory properties, depending on the given disease and organ. Leukotriene B4, for instance, is pro-inflammatory by virtue of its chemo-attractant properties for polymorphonuclear leukocytes (PMN); however, LOX-derived AA products may be further metabolized, by any of three pathways (Figure 3), to generate distinctly anti-inflammatory and pro-resolving eicosanoids called lipoxins. Briefly, the first pathway, within eosinophils, monocytes and epithelial cells, involves the stereospecific insertion of O2 at carbon C-15 of AA. Following product release from these cells and entry into either PMN cells or monocytes, a 5,6-epoxytetraene is generated by 5-LOX, which is then hydrolyzed within these recipient cells by lipoxin A4 hydrolase to produce bioactive LxA4, which when released by endothelial and epithelial cells may be transformed by leukocyte 5-LOX to generate 15-epi-LxA4 or 15-epi-LxB4.

Aspirin and COX-2 in Inflammation

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ASPIRIN, COX-2, AND OTHER PRO-RESOLVING PATHWAYS

Resolvins and docosanoids are fatty acid metabolites of the COX/LOX pathways, in which the omega-3 fatty acids docosahexaenoic acid and eicosapentaenoic acid, rather than AA, are the substrates (2, 61, 63). Resolvins and docosanoids were identified in inflammatory exudates during the resolving phase of acute inflammation and shown to be potent modulators, at nanogram doses, of inflammatory processes. Specifically, they potently inhibit PMN transendothelial migration and microglial cytokine expression and also ameliorate experimental models of dermal inflammation and leukocyte accumulation in peritonitis (63, 64). The studies on resolvin metabolism are uncovering surprising new avenues in anti-inflammation research, putting fatty acid metabolites right at the forefront of potential drug therapy. These studies are also challenging existing dogma by indicating that not all eicosanoids are detrimental to inflammation. Many therapeutic contexts are likely to benefit from this more balanced appreciation of the various roles that eicosanoids play in homeostasis and pathophysiology. To add fuel to this notion, a recent and very surprising paper has shown that eicosanoids of the lipoxin family are orally active in models of acute inflammation (65).

CONCLUSIONS AND IMPLICATIONS FOR DRUG DISCOVERY

It is becoming clear that inflammation has a number of tactically placed checkpoints that limit the magnitude and duration of the response. Defects in these endogenous anti-inflammatory pathways will arguably predispose the host to chronic inflammatory diseases. The COX and LOX pathways have both protective and pro-inflammatory roles in inflammation, with the precise function of each enzyme being dependant on the inflammatory stimulus, phase of the acute inflammatory response, and other molecules that are present to tip the balance in favor of resolving or non-resolving inflammation. Our new appreciation for inflammation-related checkpoints raises a number of important matters pertaining to anti-inflammatory drug development, and we need to be mindful that during inflammation there may be multiple endogenous pathways attempting to limit or switch off the ongoing response. These pathways must not be inadvertently altered by “anti-inflammatory” drugs. The alternative benefits from this protective biological system is the realization that factors expressed during resolution could be mimicked and used to push ongoing inflammation down a pro-resolution pathway. Such an approach would entail the development of therapies that would exert multiple effects at various phases of the inflammatory response—for example, to limit leukocyte trafficking, basion cell clearance, and help restore homeostasis to inflected stromal tissue. It is provocative to think that existing anti-inflammatory agents, such as steroids whose development was based on an entirely different strategy, may exert potent pro-resolution effects.
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