Cyclooxygenase-2–selective inhibitors: Translating pharmacology into clinical utility

BRUCE N. CRONSTEIN, MD

ABSTRACT

Anti-inflammatory agents have been used for centuries, but only in the last few decades has medical science gained insight into the complex biologic roles of the primary mediators of inflammation, the eicosanoids and their derivatives. Detailed understanding of the prostaglandins and leukotrienes provides a framework for the treatment of pain, inflammation, and fever with aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs), but these agents have exacted a substantial side effect burden. The discovery of cyclooxygenase-2 (COX-2) has guided development of rationally designed therapeutic agents that have the benefits of older NSAIDs with reduced gastrointestinal toxicity. Elucidation of the structure of COX isoenzymes has been key in the development of coxibs, the COX-2–selective subset of NSAIDs. Methods to determine the degree of COX-2 selectivity have been refined and are indispensable for comparing the relative selectivity of these agents.

This review summarizes some of the key aspects of COX biochemistry, structure, and function and the evolution of understanding the mechanism of action of COX-2–selective inhibitors. The clinical relevance of COX-1 compared with COX-2 inhibition is discussed to provide a framework upon which clinicians can better appreciate current and future therapeutic applications of coxibs.

From the New York University School of Medicine, New York.
Address correspondence to B.N.C., Professor of Medicine and Pathology, Director, Division of Clinical Pharmacology, Associate Chairman of Medicine for Research, NYU School of Medicine, 550 First Ave, New York, NY 10016; e-mail: cronsb01@med.nyu.edu.

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Plant-derived salicylates have been used traditionally by many cultures for the treatment of pain and fever. In a 1763 publication, Edmund Stone described the use of salicin-containing willow bark to treat fever in a series of patients in England.[1] The synthesis of acetylsalicylic acid in the 1890s ushered in the era of pain management with aspirin,[2] which became the most frequently used drug in the world. Many nonsteroidal anti-inflammatory drugs (NSAIDs) have been developed since aspirin was discovered: over 50 NSAIDs and over 200 aspirin-containing compounds are currently available in the United States. More than 13 million people use an NSAID daily.[3]

Despite widespread clinical use of NSAIDs for nearly a century, their mechanism of action was not understood until 1971, when it was proposed that these agents inhibit prostaglandin synthesis.[4] Cyclooxygenases are critical enzymes in the biosynthetic pathways of many bioactive compounds originating from arachidonic acid, including prostaglandins, thromboxanes, and prostacyclins. Together with the lipoxygenases, cyclooxygenase (COX) enzymes play a key role in inflammation,

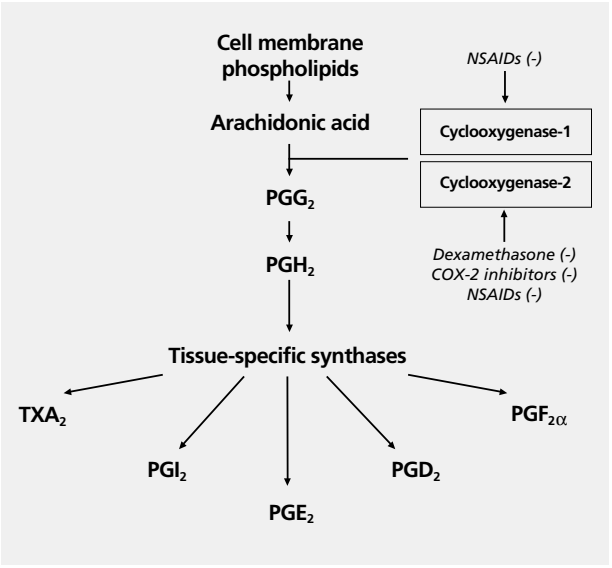

FIGURE 1. Schematic summary of the biosynthetic pathway for eicosanoids derived from arachidonic acid.[9,10]

pain, and other biologic processes. Specifically targeting these enzymes has been a major goal of drug design for the past 2 decades.

The discovery of two separate COX isoforms, COX-1 and COX-2, led to the hypothesis that the therapeutic, and conversely, adverse effects of NSAIDs lay in the specific distribution and function of each isoenzyme.[4] Inhibition of COX-1, the enzyme involved in the synthesis of prostaglandins responsible for integrity of the gastrointestinal (GI) mucosa, would lead to GI damage, while COX-2–selective inhibition should specifically alleviate pain and inflammation. This general dichotomy of action has been shown for COX-2–selective inhibitors, or coxibs, in large clinical trials for the treatment of pain and inflammation.[5,6] This review summarizes the role of COX-1 and COX-2 in prostaglandin-mediated biologic activities and the human pharmacology of selective COX-2 inhibitors, putting into clinical context the basis for the different/unique therapeutic assets of these agents.

■ EICOSANOIDS AND PROSTAGLANDINS

Milestones in eicosanoid research

In the 1930s, researchers in the United States and Sweden independently reported that compounds found in human semen had smooth muscle contraction and vasopressor properties. From their origin, von Eular called these compounds *prostaglandins*.[7] The biochemistry of prostaglandins remained elusive for 3 decades, primarily due to their paucity and instability. Elucidation of related biosynthetic pathways by Hamberg and Samuelsson in 1967 led to recognition of an abundance of biologically active compounds.[8] In 1971, Vane showed that aspirin could inhibit the synthesis of prostaglandins.[4] Aspirin is now known to target COX, a prostaglandin synthase responsible for the bicyclic endoperoxidation of fatty acids to prostaglandins. An additional pathway in eicosanoid metabolism was found to be mediated by lipoxygenases, resulting in the elucidation of the leukotriene-related pathways in the 1980s and the lipoxins in the 1990s.[9] Together, the prostaglandins, leukotrienes, lipoxins, and related compounds are known to occupy a crucial role in many biologic processes, giving the eicosanoids prominence in modern pharmacology and medicine.

Prostaglandins in physiology and pathophysiology

Eicosanoids are produced from arachidonic acid (a 20-carbon polyunsaturated fatty acid) after its liberation from the cell membrane by phospholipase in response to diverse stimuli.[10] Arachidonic acid is metabolized to eicosanoids by 2 groups of enzymes: the cyclooxygenases, which produce prostaglandins and thromboxane; and the lipoxygenases, which catalyze leukotriene and lipoxin synthesis. Eicosanoids play a key role in inflammation (**Figure 1**).[10]

The COX enzyme ultimately catalyzes the formation of prostaglandin (PG) H_2 from arachidonic acid. Within the tissues, PGH_2 is converted to a series of prostaglandins with a wide spectrum of biologic activities.[11] NSAIDs can inhibit the COX isoenzymes. Three lipoxygenases catalyze the metabolism of arachidonic acid to the leukotrienes through a series of reactions.[10] The lipoxygenases are not inhibited by NSAIDs.

Prostaglandin receptors

Prostaglandins possess diverse biologic activities and are therefore significant in the pathophysiology of a wide array of diseases. The tissue-specific and nonoverlapping properties of prostaglandins reflect the compartmentalized nature of receptors through which they act.[12] Many prostaglandin receptors are G-protein coupled receptors, designated EP, FP, IP,

TP, and DP; their cognate ligands are PGE_2, $PGF_{2\alpha}$, PGI_2, TXA_2, and PGD_2, respectively.

In light of the many activities of PGE_2, it is not surprising that 4 distinct receptor subtypes ($EP_{1\text{-}4}$) have been found to transmit signals from this molecule.[12] All 8 prostaglandin receptors have been cloned and their physiologic roles explored in receptor knock-out mice. Although there is obvious therapeutic potential in the ability to block specific activities of prostaglandins, the physiologic role of the receptors is only partially characterized, and subtype-selective antagonists remain elusive.

■ CYCLOOXYGENASES

Discovery

In 1988, the synthesis of a COX-like enzyme was shown to occur in response to interleukin (IL)-1 and bacterial lipopolysaccharide.[13,14] An induced form of COX was described that was immunologically distinct from a constitutive enzyme.[15] It was mitogenesis research, however, that led to the discovery of the COX-2 gene. In a study of gene activation in response to *src*, an mRNA expressed in Rous sarcoma virus–transformed cells was found that was homologous to COX.[16] COX-2 has been cloned from a variety of species, including humans.[17]

Similarities, differences, and interactions of COX enzymes

The ability of COX isoenzymes to orchestrate complex prostaglandin-mediated physiologic functions reflects an elaborate interplay between the 2 forms of the enzyme. Contributing to this balance are differences in their structure, level of expression, interaction with other enzymes, and feedback regulation.

In all species examined, COX-1 and COX-2 proteins have approximately 60% amino acid sequence identity.[18] The 3-dimensional structure of the COX enzymes are strikingly similar to each other.[19,20] COX isoenzymes are similar in active site structure. Both isozymes have an active site consisting of a hydrophobic channel, and amino acids in this region are nearly identical. Three amino acid differences, however, result in a larger and more accessible channel, in COX-2 (**Figure 2**).[21] Inside the hydrophobic channel of COX-2, substitution of a valine for isoleucine at residue 523 of COX-2 creates a "side pocket" that selectively allows certain

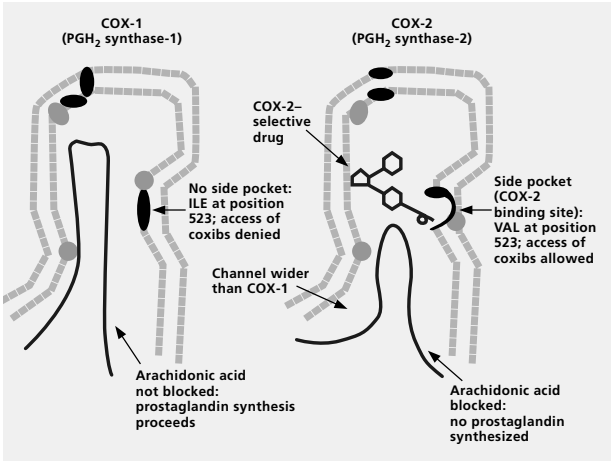

FIGURE 2. Structure of the COX-1 and COX-2 enzymes.[21] Schematic showing active site similarities and differences. ILE = isoleucine; VAL = valine.

agents to bind and inhibit this enzyme.[22]

Although the overall structure and essential catalytic activity of the 2 COX isoenzymes are similar, there are vivid distinctions in their regulation and expression. The 2 enzymes are encoded on different chromosomes, and differ in translational and post-translational regulation. In general, COX-1 is constitutive, and its expression is regulated by hormonal signals involved in maintaining physiologic homeostasis.

Consistent with the properties of "housekeeping" genes, COX-1 lacks a TATA box.[18] COX-1 is developmentally controlled, and little is known about how COX-1 expression is regulated. COX-1 is expressed in all tissues, albeit at different levels and not necessarily in all cells of a given tissue. Importantly, COX-1 but not COX-2 is constitutively expressed in the stomach, where it is involved in mucosal defense and repair.

Though COX-2 can also be constitutive in some tissues, COX-2 expression and activity is largely responsive to adverse stimuli, such as inflammation and physiologic imbalances. The COX-2 promoter has several putative regulatory regions that bind transcription factors. Although dozens of COX-2 stimulatory factors have been identified, those commonly seen in inflammation, and upregulated in the proinflammatory milieu, are key in regulation of COX-2 signaling pathways. These include transcription factors that respond to bacterial endotoxin, IL-1, and TNF-α such as NFκB, C/EBP, and protein kinases (ERK1/2 and MAPK).[23] The presence

of a cyclic adenosine monophosphate (cAMP) response element (CRE) in the COX-2 promoter may allow for COX-2 expression to be directly regulated by feedback from prostaglandins through their influence on cellular cAMP levels.[24] The presence of cytokines stabilizes COX-2 transcripts. Control of COX-2 transcription and translation is thought to be the primary mechanism by which steroids such as cortisol and dexamethasone modulate this enzyme. Post-transcriptional factors also play a role in the expression of COX-2, an immediate early gene, whose expression is controlled by mRNA splicing and translational efficiency.[18,24]

Some prostaglandin-mediated physiologic activities are carried out by only one COX isoenzyme while other activities involve both isoenzymes. For example, COX-1 is essential for thromboxane-mediated aggregation of human platelets and parturition, whereas COX-2 is essential to ovulation and nidation.[25] Other processes, such as inflammation and carcinogenesis, are mediated by both COX-1 and COX-2. In inflammation, COX-2 plays dual roles, both initiating and resolving inflammation.

Some of the segregated activities of COX-1 and COX-2 in cells simultaneously expressing both isoenzymes can be explained by the local concentration of arachidonic acid substrate. The level of enzyme expression itself also plays a role. The activity of each isoenzyme may be regulated in other ways. For example, COX-1 is subject to negative allosteric inhibition such that at lower concentrations of arachidonic acid, COX-2 may be exclusively active, despite the presence of COX-1.[23]

COX isoforms differ in their ability to interact with the terminal enzymes of prostaglandin synthesis.[23] For example, in the presence of COX-1, COX-2 appears to selectively target specific prostaglandin synthases, resulting in a shift from the production of several prostaglandins to a preferential production of PGE_2 and prostacyclin.[26]

The initial notion that COX-1 and COX-2 have unique and mutually exclusive functions has evolved to a concept incorporating multiple and complicated physiologic pathways and function. The view of COX-2 as *the inducible COX enzyme* is an oversimplification. While it is upregulated in response to certain stimuli, COX-2 is expressed constitutively in some tissues. In most tissues where COX-2 is constitutively expressed—notably the brain and kidney—the enzyme is involved in biologic response to physiologic stress. In the kidney, the macula densa is an important component of the renin-angiotensin system that orchestrates sodium balance and fluid volume by monitoring salt concentration.[27] COX-2 is constitutively expressed in the macula densa, and levels there are increased during salt deprivation, suggesting that prostaglandins produced by COX-2 are important in sodium reabsorption in response to volume contraction.[28] In the brain, prostaglandins are involved in nervous system functions such as sleep-waking cycles, fever induction, and pain transmission. While COX-2 is constitutively expressed in the brain, it is also upregulated in parallel with fever and in response to seizures.[29]

■ CYCLOOXYGENASE INHIBITORS

Pharmacologic inhibition of COX enzymes

Insight into cyclooxygenase structure and function has helped clarify the mechanisms through which NSAIDs produce their therapeutic benefits and toxicity. The different ways in which nonselective NSAIDs and coxibs, the selective COX-2 inhibitors, interact with each isoenzyme can explain many of the observed clinical effects, both good and bad, of these agents. Furthermore, this understanding has also provided the basis for a rational approach to designing safer drugs.

The "classical" nonselective NSAIDs bind to both COX-1 and COX-2, interacting with the hydrophobic channel of the COX isoenzymes. Aspirin, unlike other NSAIDs, irreversibly acetylates a serine residue in both COX-1 and COX-2 to prevent binding of arachidonic acid. Other nonselective NSAIDs compete directly for arachidonic acid, inhibiting cyclooxygenase activity in a rapid but reversible manner.[23] Although nonselective NSAIDs bind both COX-1 and COX-2, each isoform is inhibited to different degrees. Coxibs, the COX-2–selective inhibitors, preferentially bind to and inhibit COX-2. Coxibs are selective agents because they bind COX-1 poorly and in a rapidly reversible manner, whereas they bind COX-2 more tightly. This occurs in 2 stages; binding of coxibs to COX-2 during the second stage is tight, with dissociation occurring only slowly (minutes to hours). Preferential inhibition of COX-2 is thought to be due to the additional space in the COX-2 hydrophobic channel, as well as to the presence of a side pocket in the channel. This side pocket can discriminate the coxibs from nonselective agents based

on the different overall structures of these agents, in particular, by the presence in coxibs of specific side chains (**Figure 2**).[21]

COX-2 selectivity

Coxibs spare the beneficial activity of COX-1, that is, its role in the synthesis of prostaglandins important to the GI mucosa. This led to the idea that COX-1–sparing drugs are likely to be less ulcerogenic. Assays were developed in order to delineate the degree of selectivity a given NSAID may have for COX-1 or COX-2. This determination has become especially important for the newer coxibs.

There are in vitro as well as ex vivo methods to determine the 50% inhibitory concentration (IC_{50}) of various NSAIDs and coxibs for each enzyme (**Figure 3**).[30] The results of in vitro assays, which rely on recombinant enzymes, are useful for drug screening but are difficult to interpret and are sometimes contradictory. This may be due to factors like enzyme and substrate used, incubation periods, and other experimental variables. Whole-blood assays (ex vivo), which use whole blood from healthy adults, are the most widely accepted for the determination of COX selectivity.

Activity of COX-1 is determined by measuring thromboxane B_2 synthesis by platelets in whole blood. For COX-2, activity is measured as the synthesis of PGE_2 in whole blood. The use of ex vivo assays is most successful when tests are highly standardized and results are based on large numbers of subjects, as variation between individuals may be as high as 20%.[31] In addition, membrane effects and biotransformation may influence results. Another limitation of this approach is that selectivity in blood may not reflect selectivity at the mucosa. For example, whole-blood assays showed that diclofenac, the most effective COX-2 inhibitor among traditional NSAIDs, remained a potent inhibitor of prostaglandin production in gastric mucosal biopsies.[32] Use of biopsies, however, is not necessarily representative of the in vivo events, and COX enzymes may be differentially expressed in patients with ulcers compared with healthy donors used in these experiments. Although ex vivo assays identify inhibition of COX enzymes at therapeutic plasma levels, COX selectivity at the concentrations seen in the tissues remains unknown.

The IC_{50} values obtained using in vitro or ex vivo assays are expressed as a ratio of COX-1 to

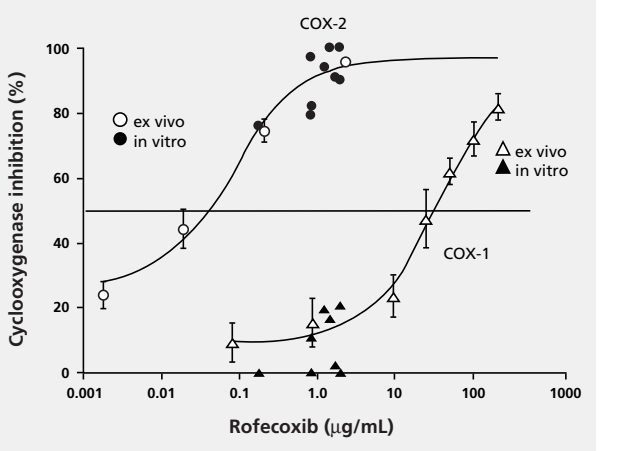

FIGURE 3. In vitro and ex vivo inhibition of cyclooxygenases by the COX-2–selective agent rofecoxib. Rofecoxib concentration-effect curves for COX-1 and COX-2 determined in vitro (filled circles and triangles), and ex vivo inhibition of COX-1 and COX-2 in patients with rheumatoid arthritis who received 50 mg rofecoxib once daily for seven days (open triangles and circles). (Adapted with permission from FitzGerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. N Engl J Med 2001; 345:433–442. Copyright © 2001 Massachusetts Medical Society. All rights reserved.)

COX-2 inhibition. As a more selective drug requires a lower concentration (IC_{50}) to be effective, the ratio for a COX-2–selective agent will be higher than 1. These pharmacologic methods have potential drawbacks that necessitate careful interpretation of the data.

Several important considerations should not be overlooked in the discussion of the pharmacology of COX inhibitors. First, the relation between the relative inhibition of COX-1 and COX-2 and alteration of prostaglandin-mediated biologic functions is not linear.[33] As pharmacologic targets, the dose-effect thresholds of efficacy and safety for COX-1 and COX-2 inhibition are probably undefinable. Even if it were possible to accurately predict the relative selectivity of COX inhibitors in vivo, it is still not known to what extent, and for how long, COX-1 can be inhibited without an increased risk of GI toxicity. Conversely, the degree of COX-2 inhibition needed to produce anti-inflammatory responses in vivo also is unknown.[31] There are currently insufficient data to accurately correlate biochemical and pharmacologic measures of COX selectivity with clinical efficacy and safety, and the question of how to determine the clinically measurable benefit of selective COX-2 inhibition remains.[3]

What is clinically relevant COX selectivity?

Clinical endpoints, ascertained through trials, are necessary to determine whether COX-2–specific inhibition translates to efficacy and greater GI safety. Various clinical endpoints have been employed for this purpose. GI symptoms, such as dyspepsia, are poorly correlated to gastric lesion formation, and the role of COX-1 in these events is unclear.[33] Endoscopic data are more favorable, and a baseline and post-treatment comparison should provide strong evidence for ulcerogenesis in a clinical setting.[31] Endoscopic studies remain a surrogate for outcomes studies, which should be sought after as the definitive arbiters of GI safety as well as analgesic/inflammatory efficacy.

COX-2–selective inhibitors

In light of the collective evidence for COX selectivity, only a few drugs have a COX-1/COX-2 ratio suggesting that limited inhibition of COX-1 would occur at therapeutic levels. Three drugs that have existed for some time—meloxicam, nimesulide, and diclofenac—all have a COX-1/COX-2 ratio in the range of 10 to 30.[33,34] These drugs, while preferentially inhibiting COX-2, have considerable COX-1 inhibitory activity. Meloxicam, nimesulide, and diclofenac show significant inhibition of COX-1 at therapeutic levels.[35–37] Furthermore, large clinical trials have not been able to show a substantial GI benefit with these agents.[30]

Two drugs approved by the US Food and Drug Administration, rofecoxib and celecoxib, have been shown to have the greatest selectivity for COX-2. In vitro and ex vivo studies show that these coxibs have COX-1/COX-2 ratios that are 10- to 100-fold greater than existing nonselective NSAIDs.[21,33,38] Furthermore, ex vivo assays following single doses in normal hosts showed negligible (~10%) inhibition of TXB_2 release by platelet COX-1. Unlike rofecoxib, however, celecoxib inhibited release of TXB_2 in a dose-dependent manner and had an interindividual variation in response that ranged from 10% to more than 80% inhibition.[39] Both rofecoxib and celecoxib have been examined in clinical trials large enough to have sufficient statistical power for detection of clinical specificity.[5,6] The results fulfilled expectations that COX-2–specific inhibitors could achieve efficacy equal to nonselective NSAIDs with less GI toxicity (see article by Scheiman, this supplement). Additional COX-1–sparing drugs (etoricoxib, valdecoxib, and COX-189; see article on the development of coxibs in this supplement) are in preclinical and clinical development. The outcomes of clinical trials evaluating coxibs are discussed in detail in a recent review.[40]

■ CONCLUSIONS

The magnitude of NSAID use, the high incidence of gastropathy in NSAID users, and the significant morbidity and mortality of NSAID-associated GI outcomes underscore the need for less toxic NSAIDs. In a single decade since the discovery of COX-2, a deeper appreciation of the complexity of prostaglandin metabolism has emerged, leading to new therapeutic avenues capable of overcoming the limitations of classical NSAID toxicity. Despite the challenges of defining COX selectivity, the paradigm that COX-1–sparing drugs are safer has successfully guided the development of promising new anti-inflammatory agents. Patient variability, pharmacodynamics, and preexisting risk factors influence COX-2–specificity, which is why it has been imperative to show COX specificity in large clinical trials with adequate numbers of patients and events. Clinical trials convincingly show that agents specifically inhibiting COX-2 are equivalent in efficacy to nonselective NSAIDs and have a lower incidence of GI toxicity. Although clinical specificity of COX-2 inhibitors has been shown, there is still much not known about COX-1 and COX-2 across the spectrum of health and disease. Intimate knowledge of the pharmacology of COX-2 inhibitors in health and disease will likely open the door to new clinical applications for these drugs.

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