The COXIB Experience: A Look in the Rearview Mirror

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Key Words

cyclooxygenase, NSAID, COXIB, inflammation, gastrointestinal toxicity, cardiovascular toxicity

Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used prescription and nonprescription drugs in the world. The discovery of cyclooxygenase (COX) as the target of NSAIDs, the subsequent identification of two isoforms of COX (COX-1 and COX-2), and studies of their regulation and sites of expression led to the hypothesis that COX-2 is the molecular target for the anti-inflammatory and analgesic effects of NSAIDs. A corollary was that COX-2-selective inhibitors (COXIBs) would retain the desirable effects of NSAIDs without some of their liabilities (e.g., gastrointestinal toxicity, which was ascribed to COX-1 inhibition). The first marketed COXIBs exhibited reduced gastrointestinal side effects relative to traditional NSAIDs and were enormous commercial successes. However, clinical trials testing the hypothesis that COXIBs prevent recurrence of premalignant colon polyps uncovered adverse cardiovascular effects that are mechanism based. This review provides an overview of the discovery, development, and difficulties of the COXIBs, a perspective on what has been learned, and speculation on the way forward.

DISCOVERY OF NSAIDS AND THEIR TARGET

Cyclooxygenase (COX): enzyme that catalyzes the oxygenation of polyunsaturated fatty acids to prostaglandin endoperoxides. Also known as PGG/H synthase

Prostaglandins (PGs): bioactive lipids that mediate inflammation, pain, platelet activation, vascular tone, cell proliferation, angiogenesis, etc.

NSAID: nonsteroidal anti-inflammatory drug

PGI_2: prostaglandin I_2 (prostacyclin)

TxA_2: thromboxane A_2

PPAR: peroxisome proliferator activated receptor

The history of nonsteroidal anti-inflammatory drugs (NSAIDs) traces to the dawn of civilization and is an amalgam of natural products isolation, medicinal chemistry, and clinical exploitation. Advances in the discovery of NSAIDs paralleled the creation of new scientific disciplines (e.g., chemistry, pharmacology, toxicology), and technological advances in these emerging fields led to new developments in the discovery and development of NSAIDs. A fascinating and entertaining look at the evolution of the major lines of anti-inflammatory agents has been written by Brune and Hinz, which highlights the importance of serendipity as well as trained observation in the early discovery of active compounds (1). **Figure 1** summarizes the evolution of some of the major classes of NSAIDs prior to the discovery of cyclooxygenase-2 (COX-2). The first three classes of NSAIDs were discovered without the benefit of animal models of efficacy and were based on direct human testing. Pharmacology and toxicology played a minimal role in the introduction of salicylic acid, aspirin, phenacetin, propyphenazone, and phenylbutazone. However, shortly after World War II, the guinea pig erythema and rat foot pad edema models of inflammation were developed, which provided preclinical testing grounds for new compounds. This led to the introduction of oxyphenylbutazone, the oxicams, the fenamates, diclofenac, indomethacin, sulindac, and the profens. At approximately the same time, Brodie & Axelrod (59) and Smith & Williams (60) established that phenacetin is metabolized to acetaminophen, which represents an active metabolite responsible for the analgesic and antipyretic activities of phenacetin. Subsequent to this intense post-war period of discovery and clinical development, Vane and colleagues (2, 2a, 2b) discovered that NSAIDs inhibit the production of prostaglandins (PGs) from polyunsaturated fatty acids by inhibiting the first enzyme in the biosynthetic cascade, i.e., cyclooxygenase (COX). Three reports published in succession (in 1971) established COX as an important molecular target for mechanistic studies and a focus for new screens for candidate NSAIDs (2, 2a, 2b).

COX catalyzes the double dioxygenation of arachidonic acid to prostaglandin endoperoxides, PGG_2 and PGH_2, (**Figure 2**) and for this reason also is called prostaglandin G/H synthase. The oxygenation of arachidonic acid occurs at the cyclooxygenase active site, and the reduction of PGG_2 to PGH_2 occurs at the peroxidase active site. Both active sites are present on the COX protein, although on opposites sides of the molecule. The heme prosthetic group connects the two active sites spatially and functionally. NSAIDs bind at the cyclooxygenase active site and prevent binding of arachidonic acid.

The major control point in PG biosynthesis is release of arachidonic acid substrate from phospholipid stores following cell stimulation. Treatment of cells or tissues with a broad range of agonists or physical stimuli causes activation of phospholipases that release arachidonic acid; arachidonate is oxygenated by COX to PGH_2. PGH_2 diffuses from the COX protein and is converted by multiple isomerases or a reductase to one of five different final metabolites. These are PGE_2, PGD_2, $PGF_{2\alpha}$, prostaglandin I_2 (PGI_2), and thromboxane A_2 (TxA_2) (**Figure 2**). Most tissues convert PGH_2 to a few of these final products but not all five. For example, blood platelets make TxA_2 and PGD_2, and vascular endothelium makes PGI_2 and PGE_2.

A complex panel of receptors for PGs and TxA_2 exists that comprises a family of G protein–coupled receptors and their splice variants. In addition, PGI_2 is a ligand for the nuclear receptor peroxisome proliferator activated receptor-delta ($PPAR_\delta$). Animals bearing targeted deletions for many of these receptors have been generated, and they have helped to define the roles of the individual PGs and TxA_2 in a range of physiological and pathophysiological responses. It seems remarkable that NSAIDs, which inhibit the first step in this complex cascade, are useful pharmacological agents given the complex signaling networks they interrupt. But their beneficial effects in humans afflicted with inflammation, pain, and fever have been documented for millennia.

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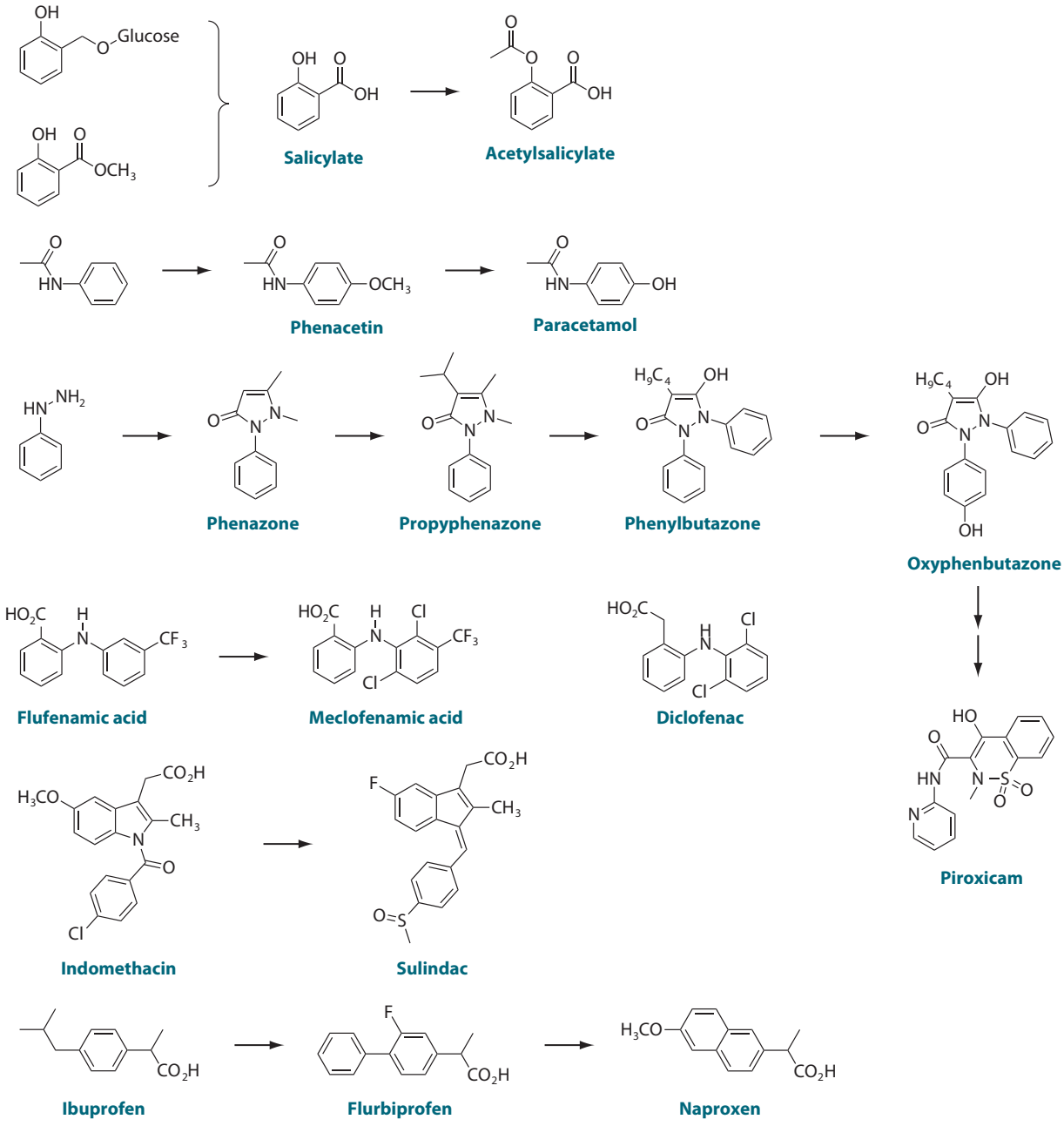

Figure 1

Evolution of major classes of NSAIDs prior to discovery of COX-2.

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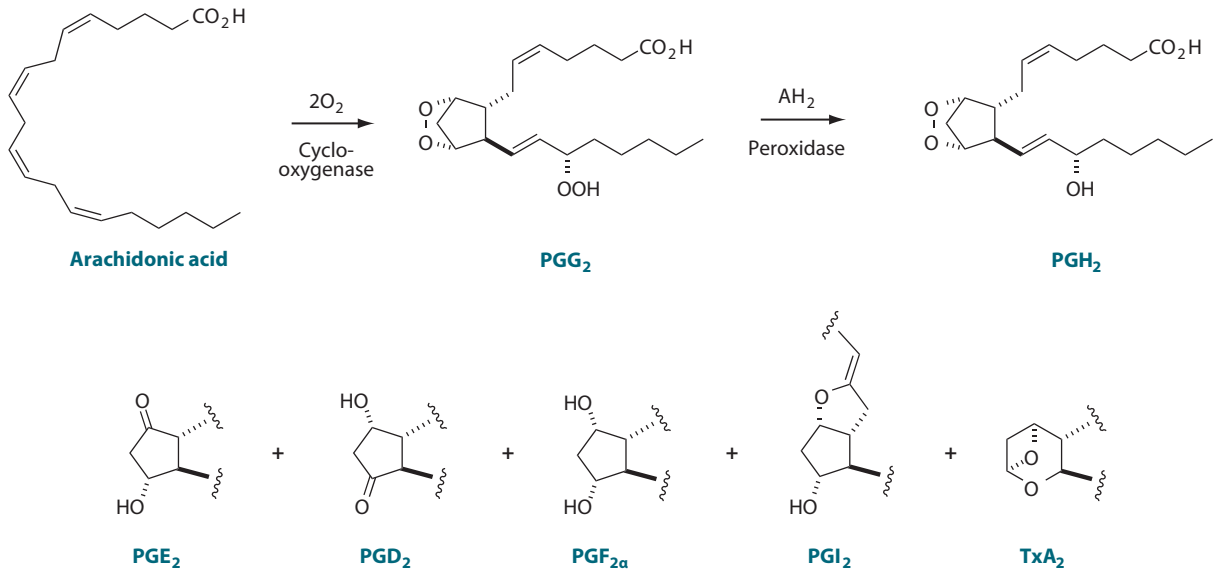

Figure 2

Oxygenation of arachidonic acid to prostaglandin endoperoxides and ultimate products of PGH_2 metabolism.

Likewise, a range of hazardous side effects, including liver, kidney, gastrointestinal, and central nervous system toxicity, has been associated with the use of NSAIDs. Some of the side effects are related to COX inhibition, whereas others are not. In addition, some interesting off-target effects have been reported for certain NSAIDs that represent potential targets for further development. The concentrations required to engage these non-COX targets are frequently higher than those that inhibit COX in vivo, so their utility is limited by mechanism-based, COX-dependent side effects.

The realization that COX is a major molecular target for NSAID action was a key insight into the mechanism of action of anti-inflammatory agents and related compounds. It underscored the importance of the arachidonic acid cascade, in particular the production of PGs and TxA_2, in the development of inflammation. Given the contribution of chronic inflammation to the etiology of a range of diseases, this discovery had potential implications for their treatment. However, it did not provide a strategy to reduce the mechanism-based side effects of NSAIDs. Because all PGs were believed to originate from the action of a single COX protein, inhibition of COX in different tissues would lead to desired pharmacological effects as well as undesired side effects, e.g., gastrointestinal toxicity and renal toxicity. The frequency and magnitude of these side effects vary greatly with different individuals, and an indeterminate number of patients are so sensitive to the gastrointestinal side effects that they are unable to take the drugs at all.

DISCOVERY OF COX-2 AND THE COX-2 HYPOTHESIS

COX was purified in 1976 and its cDNA cloned in 1988 (3, 4). Analysis of protein and mRNA levels indicate that the enzyme is widely distributed in mammalian tissues and is, for the most part, constitutively expressed. However, early reports indicated (*a*) a massive increase in PG biosynthesis in baby hamster kidney fibroblasts following transformation with polyoma virus; (*b*) delays in PG production of several hours following agonist stimulation of cells; (*c*) differential inhibition of basal and induced PG biosynthesis by anti-inflammatory steroids; and (*d*) significantly different

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IC_{50}s for inhibition of PG synthesis by certain NSAIDs in different tissues. These observations were inconsistent with the simple picture of a single constitutively expressed COX enzyme that produced PGs in response to agonist stimulation and phospholipase activation. They were clarified with the discovery of COX-2, which was played out in reports from 1990 to 1993.

The discovery of COX-2 resulted from the complementary efforts of multiple research groups working in very different areas and using very different approaches (for a more detailed account, see Reference 5). Key findings included (*a*) discovery of two different COX mRNAs and proteins, one of which is constitutively expressed and unaffected by glucocorticoid treatment, the other of which is induced by cytokines or lipopolysaccharide and its induction inhibited by glucocorticoids; (*b*) cloning of a new COX gene induced by transformation of chicken embryo fibroblasts by Rous sarcoma virus or stimulation of murine or human fibroblasts by phorbol esters; and (*c*) heterologous expression of the new COX gene and demonstration that it possesses both cyclooxygenase and peroxidase activities. The new COX protein (COX-2) is approximately 60% identical to the previously known COX (COX-1), and exhibits a very different pattern of regulation and tissue distribution (6). In contrast to the rather widespread constitutive expression of COX-1, COX-2 is expressed constitutively in a rather narrow range of tissues but is strongly inducible in response to treatment of cells with an extremely broad range of agonists, including cytokines, growth factors, tumor promoters, viruses, bacterial lipopolysaccharides, and laminar flow stress inter alia.

The inducibility of COX-2, particularly in macrophages treated with cytokines, and its inhibition by anti-inflammatory steroids suggested that COX-2 is the true molecular target for the anti-inflammatory effects of NSAIDs. Furthermore, the presence of COX-1 in the gastrointestinal tract gave rise to what might be termed the full COX-2 hypothesis—that COX-2 inhibition is responsible for the anti-inflammatory effects of NSAIDs, whereas COX-1 inhibition is responsible for some of their undesired side effects, specifically gastrointestinal toxicity (**Figure 3**). The latter part of the hypothesis was consistent with the fact that existing NSAIDs inhibited both COX enzymes to varying extents (7). It led to the prediction that a COX-2-selective inhibitor

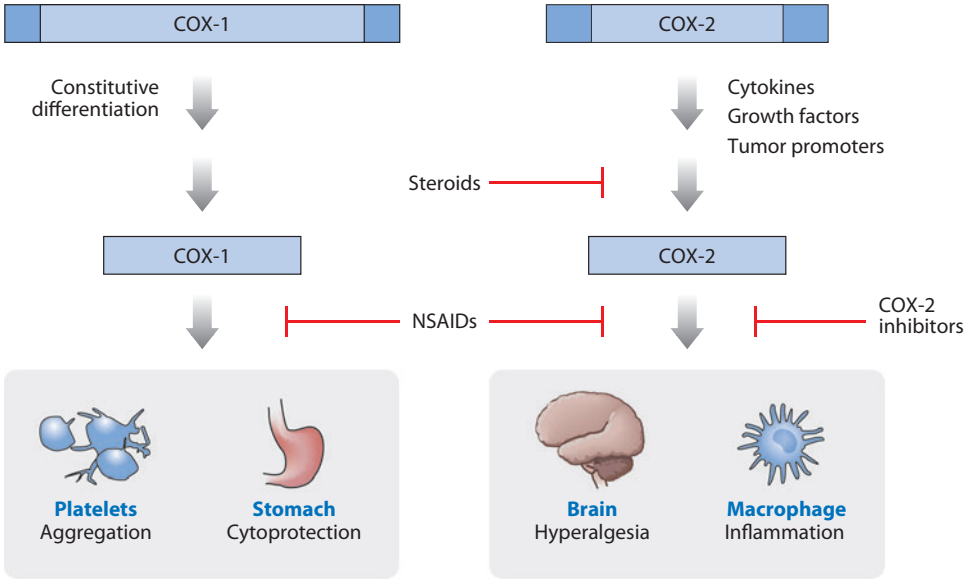

Figure 3

Regulation and inhibition of cyclooxygenases.

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(COXIB) would exhibit anti-inflammatory (and possibly analgesic and antipyretic) activity without the gastrointestinal toxicity exhibited by traditional NSAIDs. The COX-2 hypothesis was extremely attractive because of the extensive clinical history of NSAIDs that had identified an unmet medical need and the obvious role of COX inhibition in both the beneficial effects and undesired side effects of NSAIDs. Thus, it appeared that COX-2 was a highly validated target for drug discovery, and a number of companies aggressively pursued efforts to develop selective inhibitors. Such compounds were designed to extend the benefits of NSAIDs to individuals who could not take them because of sensitivity to their gastrointestinal side effects.

COXIB: a COX-2-selective inhibitor

Diarylheterocycle: class of COXIB comprised of two aromatic groups attached to a central heterocyclic functionality

DEVELOPMENT OF THE COXIBS

The discovery of COXIBs and their clinical development in a relatively short time after the discovery of COX-2 is a tribute to the power of medicinal chemistry coupled with highly focused efforts to exploit a validated target. The existence of multiple different NSAID scaffolds that inhibited both COX enzymes provided a basis for efforts to introduce selectivity. The scaffold that resulted in the first drugs to market was a diarylheterocycle, which is an offspring of the phenylbutazone lineage. Dup697 is a diarylthiophene with a methylsulfone in one aromatic ring that was reported to inhibit PG synthesis in macrophages but not in platelets. It also exhibited reduced GI toxicity in rodent models (8). These were puzzling observations at the time, but their interpretation became obvious with the discovery of COX-2 and the demonstration of differential enzyme distribution between the two cell types, i.e., COX-1 in the platelet, COX-2 in the macrophage. Extensive structure-activity studies revealed the key determinants for COX-2 selectivity—two aromatic rings attached to a heterocycle or carbocycle and a sulfonamide or sulfone substituted on the *para* position of one of the rings (**Figure 4**) (9, 10). Celecoxib and valdecoxib are derived

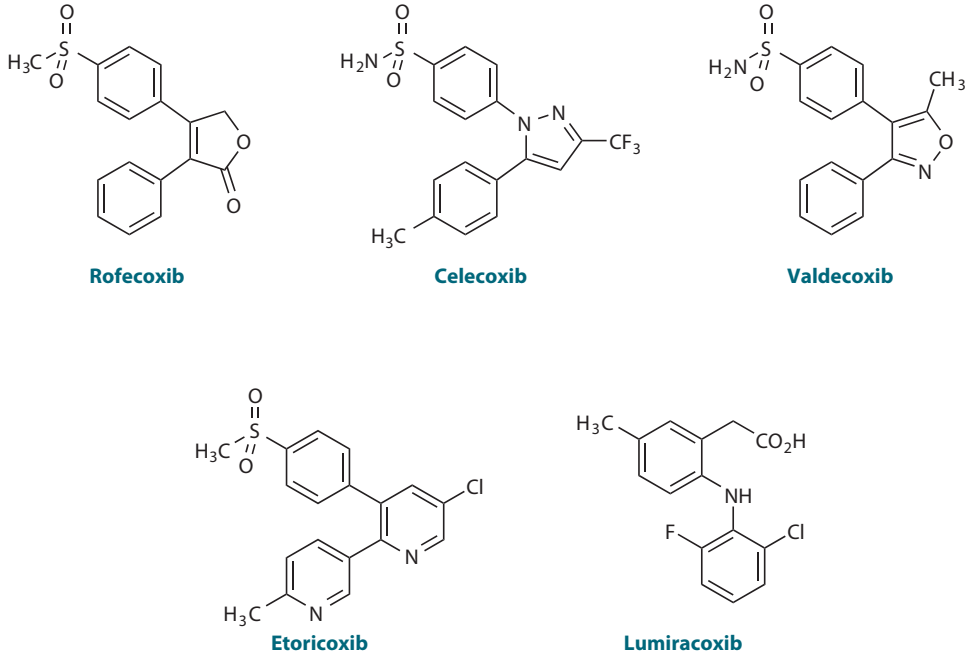

Figure 4

Structures of COXIBs.

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from the sulfonamide subclass but contain pyrazole and oxazole heterocycles, whereas rofecoxib and etoricoxib represent the sulfone subclass but contain furanone and pyridine heterocycles.

Other existing NSAID scaffolds probed as sources for COX-2-selective inhibitors included the acidic sulfonamides related to nimesulide, the β-ketoenols related to meloxicam, and the arylacetic acids or arylpropionic acids related to diclofenac, indomethacin, or flurbiprofen. Attempts also were made to develop a COX-2-selective covalent modifier related to aspirin. The only nondiarylheterocycle scaffold that resulted in a new marketed drug was an arylacetic acid evolved from diclofenac (**Figure 4**) (11). However, detailed analysis revealed that several marketed NSAIDs have reasonable COX-2 selectivity when evaluated in ex vivo assays (see below).

STRUCTURAL BASIS OF COX INHIBITION

COX is a hemeprotein that is a dimer of 70 kDa subunits (12). The homodimer is membrane bound and is localized in the lumen of the endoplasmic reticulum as well as the nuclear envelope (**Figure 5*a***). There are reports of COX-2 expression in other cellular compartments in some tumor cells (e.g., lipid bodies). Some evidence exists for the formation of a heterodimer of COX-1 and COX-2 subunits but this needs further biochemical confirmation. The two active sites exist in different locations of the 70 kDa COX subunit. The cyclooxygenase active sites are highly homologous. Although the overall identity in a given species of COX-1 and COX-2 is approximately 60%, the identity in the active site is higher (~85%), which limits the possible interactions that can give rise to selectivity. The active sites are located at the terminus of a long channel that runs from the membrane-binding domain to the interior of the protein. The membrane proximal region is separated from the active site by a gate that must open to permit substrate or inhibitor binding. All substrates and COX inhibitors, whether nonselective or selective, bind in the active site above the gate (**Figure 5*b***). One of the gate residues, Arg-120, provides a positive charge that binds the negative charges of carboxylic acid substrates and inhibitors (e.g., salicylates, profens, indoleacetic acids).

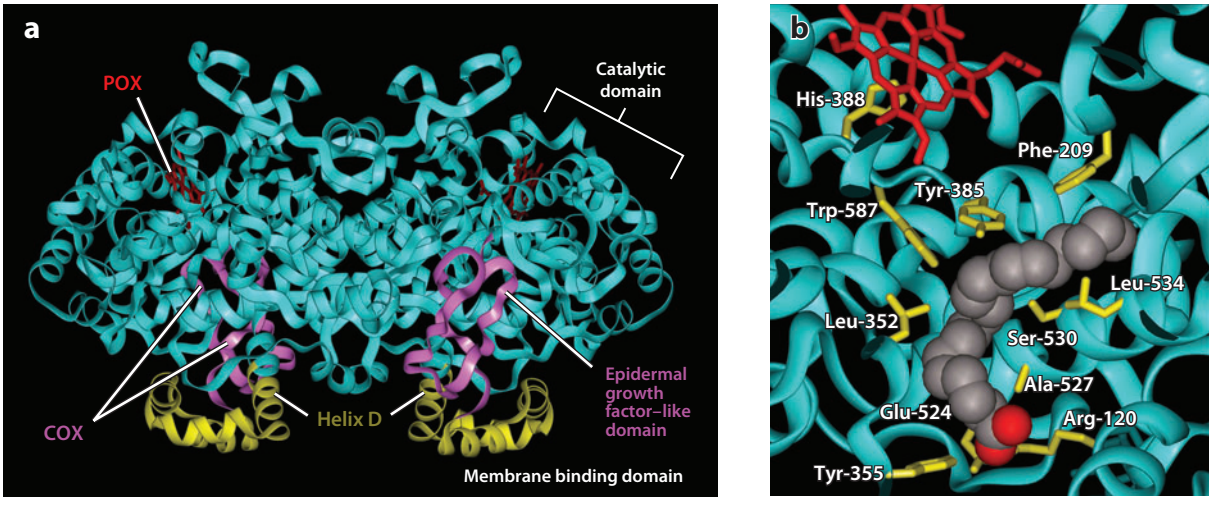

Figure 5

COX-2 structure. (*a*) Structure of homodimeric protein with major functional domains and active sites highlighted. (*b*) Cyclooxygenase active site with molecule of arachidonic acid bound. Side chains of active site residues illustrated in yellow. Arachidonic acid colored gray with carboxylic acid oxygens colored red. Reproduced with permission from (58).

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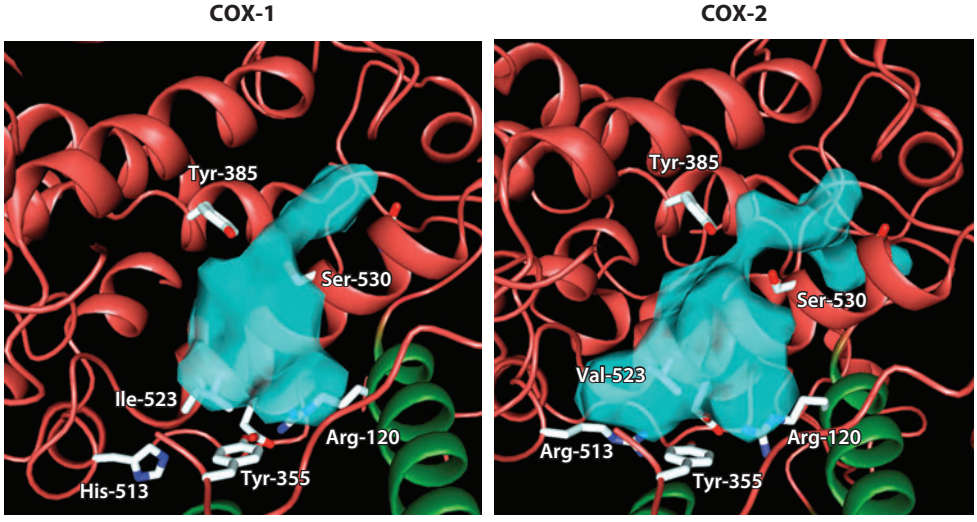

Figure 6

Comparison of cyclooxygenase active sites of COX-1 and COX-2. Solvent-accessible space highlighted in blue. Reproduced with permission from (58).

Despite the high homology between the two enzymes, the COX-2 active site is larger by approximately 27%. This is due to a side pocket off the main active site channel, which is more accessible in COX-2 than in COX-1 (**Figure 6**). The side pocket is bordered by a Val residue (Val-523) in COX-2 but an Ile residue in COX-1 (Ile-523). This subtle difference enables tighter binding of the sulfonamide or sulfone group of the diarylheterocycles celecoxib and rofecoxib in the side pocket of COX-2. Mutagenesis of Val-523 of COX-2 to Ile renders the mutant COX-2 resistant to celecoxib and rofecoxib. Another conserved difference in the side pockets of the COX enzymes is Arg-513 in COX-2, which is His in COX-1. This difference at position 513 contributes somewhat to the COX-2 selectivity of the diarylheterocycles, but is a more significant factor in the ability of COX-2 to utilize amide and ester derivatives of arachidonic acid as substrates (see below).

Diclofenac and lumiracoxib are anilino-arylacetic acids that bind in an inverted fashion in the COX-2 active site (**Figure 7**). Their carboxylic acids do not bind to Arg-120 but are chelated by Tyr-385 and Ser-530; one of the chlorines of the dihaloaryl ring binds in a depression in the side of the active site channel comprised of Val-349, Ala-527, Ser-530, and Leu-531. The methyl group of the arylacetic acid functionality projects into the active site and sits adjacent to Leu-384. In ovine COX-1, the secondary shell residues behind Leu-384 are bulky and restrictive (Ile-525 and Phe-503). In human and mouse COX-2, these residues are Val-525/Leu-503 and Leu-525/Leu-503, respectively. These are smaller, less restrictive residues that allow Leu-384 some flexibility and permit the 5′-methyl group of lumiracoxib to insert into the resulting groove. This interaction is a major contributor to the greater COX-2 selectivity of lumiracoxib compared with diclofenac.

KINETIC BASIS OF INHIBITION: THE QUESTION OF SELECTIVITY

It has been known for more than 30 years that certain COX inhibitors are slow, tight-binding inhibitors (13). Detailed evaluation of the kinetics of inhibitor binding for multiple classes of inhibitor scaffolds reveals the occurrence of multiple equilibria that connect different enzyme-inhibitor

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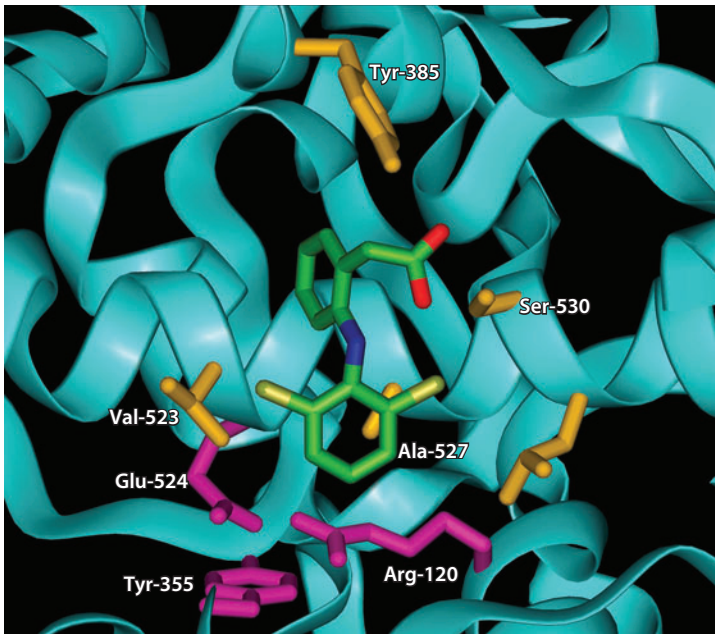
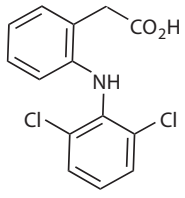
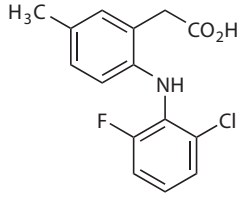




Diclofenac



Lumiracoxib

Figure 7

Structures of diclofenac and lumiracoxib with structure of COX-2-diclofenac complex. Note the carboxyl group of diclofenac does not ion-pair to Arg-120 but H-bonds to Tyr-385 and Ser-530. Reproduced with permission from (14).

complexes (Equation 1). The stability of the individual complexes is reflected in the relative rates connecting them. Only one NSAID-COX complex is covalent—that of the acetyl enzyme formed by aspirin acetylation. The remaining enzyme-inhibitor complexes are noncovalent, but some are so stable that they behave as if they are functionally irreversible.

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E - I \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E - I^*. \qquad 1.$$

The problem of selectivity and how to measure it was a major uncertainty in COXIB drug discovery and remains at the heart of the key clinical issues that the field faces going forward. The simplest kinetic behavior is displayed by compounds like ibuprofen and mefenamic acid, which appear to be simple competitive inhibitors. The kinetics of inhibition correspond to a single rapidly reversible equilibrium between free and bound inhibitor. However, this is deceptively simple kinetic behavior. The rate constant for inhibition is slower than one might anticipate for diffusion-limited association between inhibitor and protein, and the crystal structure of a COX-1-ibuprofen complex reveals that ibuprofen binds in the active site above the gate separating

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Whole blood assay: ex vivo assay for COX inhibitor selectivity in which platelets are a source of COX-1 and macrophages are a source of COX-2

it from the membrane-proximal lobby. Thus, ibuprofen must associate with the enzyme and then move through the gate to inhibit the enzyme. The rates of initial inhibitor association with the enzyme and its movement into as well as release from the active site must be of such magnitudes as to make the overall association and dissociation kinetics appear to be a single step. Absolute determination of the rate constants for each of the steps in the overall inhibition process has not been possible for any inhibitor and is complicated by the fact that the rate of inhibition is comparable to the rate of oxygenation of substrate, which causes overlap of competing steps.

Classic slow, tight-binding inhibitors such as indomethacin exhibit a second, much slower step that increases the tightness of binding by orders of magnitude. The rate of this second step varies dramatically for different classes of inhibitors as does the reverse step. For example, indomethacin binds so tightly that it appears functionally irreversible even in the presence of large excesses of substrate. In contrast, lumiracoxib is much less tightly bound and appears rapidly reversible following addition of excess substrate. For several classes of inhibitors, the molecular determinants of tight binding have been identified, and these are summarized in a recent review (14).

Diarylheterocycles such as celecoxib and rofecoxib also are slow, tight-binding inhibitors. Detailed kinetic analysis of their binding utilizing fluorescence quenching techniques reveals a more complex pattern of behavior than, for example, indomethacin. The initial rapid association of the diarylheterocycle is followed by two slow steps, the second of which is associated with the development of COX-2 selectivity. As described above, selectivity is due to binding of the sulfone or sulfonamide in the side pocket of COX-2.

Because the most potent and selective COX-2 inhibitors are slow, tight-binding compounds, kinetic assays were designed to select for them. This meant that preincubation times of 5 to 30 min were utilized in which inhibitor was incubated with enzyme in the absence of substrate. This enabled stable COX-inhibitor complexes to develop, so such preincubations were routinely incorporated. In addition, saturating substrate concentrations ($>5 \times K_m$ for arachidonate, typically, 50 μM) were employed in some cases to minimize or eliminate competitive inhibition. Thus, most of the compounds that were developed as COX-2-selective inhibitors were slow, tight binders. However, although they were selected using screens for slow, tight binding, most of the inhibitors also display some nonselective competitive inhibition of COX-1, especially at low substrate concentration (15). This was first demonstrated for diarylheterocycles but has been extended to other inhibitors when detailed kinetic analysis of inhibition has been performed (16). Thus, the COX-2 selectivity of a given inhibitor can change depending on the assay conditions, especially when low substrate concentrations are employed.

The effective arachidonic acid concentrations in most cells are uncertain and variable, so even weak competitive inhibitors can inhibit COX-1 to some extent. Also, the off-rates for different classes of inhibitors vary significantly, and detailed analysis reveals that the off-rates (k_{-2}) are much more important determinants of potency and selectivity than the on-rates. Thus, there has been serious uncertainty in extrapolating the results of in vitro inhibitor assays to in vivo COX inhibition. In addition, most potent COX inhibitors are highly bound to plasma proteins, which reduces the effective concentration of inhibitor.

The most widely used predictive assay for COX-2 selectivity in vivo is the human whole blood assay. This was originally developed as a way to monitor COX inhibition ex vivo following in vivo dosing of drugs. It has been adapted as a purely ex vivo assay in which blood samples are used as a source of platelet COX-1 and macrophage COX-2. In a more recent modification, human lung cancer (A549) cells are added to whole blood as a more reliable source of COX-2 (17). The various whole blood assays provide an estimate of potency and selectivity of inhibition of the two COX enzymes in the context of plasma protein binding. **Table 1** lists some IC_{50} and IC_{80} values for NSAIDs and COXIBs determined in the whole blood assay. A general dampening of selectivity

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Table 1 Potencies of all compounds tested as inhibitors of prostanoid formation determined in the COX-1 assay and WHMA-COX-2

	COX-1		WHMA-COX-2		IC_{50} Ratios	IC_{80} Ratios
Compound	IC_{50}, μM	IC_{80}, μM	IC_{50}, μM	IC_{80}, μM	WHMA/COX-1	WHMA/COX-1
Aspirin	1.7	8.0	7.5	30	4.4	3.8
Diclofenac	0.075	1.0	0.020	0.23	0.3	0.23
Flufenamate	3.0	80	n.d.	n.d.	n.d.	n.d.
Flurbiprofen	0.075	1.0	0.77	51	10	51
Ibuprofen	7.6	58	20	150	2.6	2.6
Indomethacin	0.013	0.46	0.13	2.0	10	4.3
Ketoprofen	0.047	1.0	0.24	6.0	5.1	6.0
Meclofenamate	0.22	3.0	0.2	1.0	0.91	0.3
Naproxen	9.3	110	35	330	3.8	3.0
Piroxicam	2.4	15	0.17	7.0	0.1	0.47
Sulindac Sulfide	1.9	3.8	1.21	11	0.64	0.29
Celecoxib	1.2	28	0.34	3.0	0.3	0.11
Meloxicam	5.7	22	0.23	2.0	0.040	0.091
Rofecoxib	63	>100	0.31	5.0	0.0049	<0.05
Sodium Salicylate	4956	49000	482	45000	0.10	0.92

Shown are potencies (μM IC_{50} and IC_{80} values) of compounds against COX-1 and William Harvey Modified Assay for COX-2 (WHMA-COX-2). Selectivities of compounds toward COX-1 were determined as IC_{50} and IC_{80} ratios for WHMA-COX-2/COX-1. n.d., not done (adapted from 17).

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ratios is observed for values determined in the whole blood assay compared with those reported using purified enzymes or microsomal fractions. Also, some rather surprising changes in rank order selectivity are observed. For example, lumiracoxib is the most selective COX-2 inhibitor in the whole blood assay even though it is a modestly potent, rapidly reversible inhibitor against purified COX-2. Conversely, celecoxib displays much lower selectivity for COX-2 in the whole blood assay compared with its selectivity against extracts from insect cells expressing recombinant enzymes. In fact, celecoxib appears comparable to the NSAID diclofenac in the whole blood assay, a result that correlates to the results of gastrointestinal toxicity in clinical testing (see below). The data in **Table 1** are useful for estimating the pharmacodynamic and toxicodynamic effects of the various compounds when combined with the plasma levels and half-lives of the individual agents (18).

VALIDATION OF THE COX-2 HYPOTHESIS

The hypothesis that COX-2-selective inhibitors are anti-inflammatory but not ulcerogenic was validated by the demonstration that COXIBs such as NS-398 and SC-58125 inhibit PG production induced by carageenan in either the rat air pouch or the rat paw but not the basal production of PGs in the stomach (19). Both compounds inhibit inflammation in the carageenan-induced models, but neither compound induces gastric lesions at doses well above those at which they inhibit inflammation. In contrast, the nonselective inhibitor indomethacin inhibits PG production in both inflamed tissues and the stomach, inhibits inflammation in the air pouch and foot pad, and induces gastric lesions at doses comparable to anti-inflammatory doses.

Interestingly, the validation of the COX-2 hypothesis observed with COX-2-selective inhibitors is not recapitulated with COX-2 or COX-1 knockout mice (20–22). Neither class of knockout animals develops gastrointestinal lesions and neither exhibits a difference in anti-inflammatory

responses to a broad range of stimuli. Reduced liver toxicity triggered by lipopolysaccharide is observed in COX-2 knockout mice, but there is no difference in carrageenan-induced foot pad edema, phorbol ester-induced skin inflammation, or arachidonic acid-induced skin inflammation (20). Likewise, reduced arachidonic acid-induced skin inflammation is observed in COX-1 knockout mice, but no difference is observed in phorbol ester-induced skin inflammation (22). The only moderately altered sensitivities of COX-1 or COX-2 knockout animals to inflammatory stimuli or to their lack of development of gastrointestinal lesions is not attributable to compensatory PG synthesis by the remaining COX enzyme. Thus, the mechanisms of the pharmacological and toxicological activities of NSAIDs appear more complex than one might anticipate by comparison to COX knockout animals.

Myocardial infarction: heart attack arising from disruption of blood flow to a region of the heart

VIGOR: Vioxx Gastrointestinal Outcomes Research

The main pathology observed in COX-2 knockout mice is impaired postnatal renal development, which does not appear similar to the renal toxicity induced by chronic NSAID treatment. In addition, cardiac fibrosis is observed in the COX-2 knockout animals, and reproductive deficits are observed in both COX-1 and COX-2 knockout animals. Importantly, no phenotypes were observed in COX-2 knockout animals that presaged cardiovascular toxicity leading to myocardial infarction.

CLINICAL TESTING OF THE COX-2 HYPOTHESIS

The Vioxx Gastrointestinal Outcomes Research (VIGOR) trial compared a single daily dose of 50 mg rofecoxib with twice daily doses of 500 mg naproxen in 8076 patients with rheumatoid arthritis. The dose of rofecoxib was twice the dose that ultimately was prescribed for anti-inflammatory activity, but was a dose used on a short-term basis for pain relief. After 11 months of follow-up, rofecoxib and naproxen were equally efficacious against rheumatoid arthritis in the VIGOR trial, but rofecoxib was associated with approximately half the number of serious upper gastrointestinal events than was naproxen (**Figure 8**) (23). The overall safety rates of the two drugs as judged by deaths from all causes were comparable. Although the death rates from cardiovascular events were similar in both groups, naproxen was associated with a significantly lower number of cardiovascular

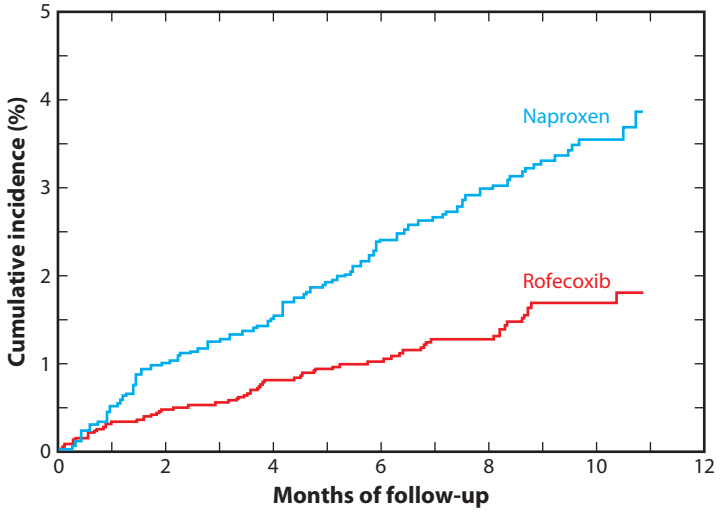

Figure 8

Cumulative incidence of gastrointestinal lesions induced by naproxen and rofecoxib in the VIGOR study. Reproduced with permission from (23).

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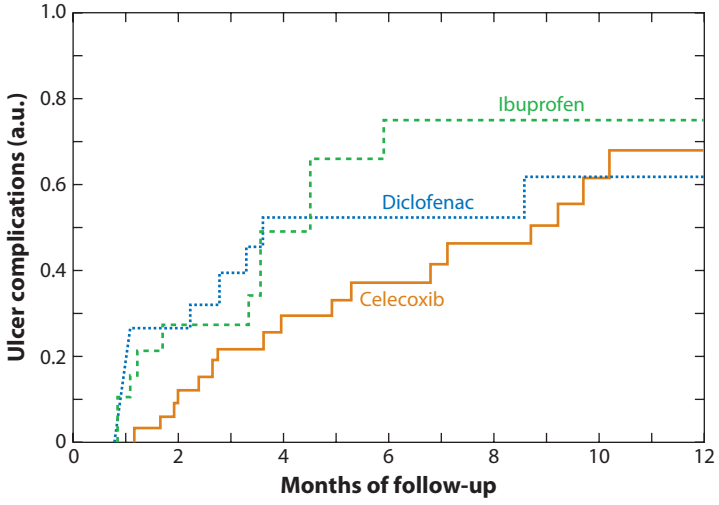

Figure 9

Cumulative incidence of gastrointestinal lesions induced by ibuprofen, diclofenac, and celecoxib in the Celecoxib Long-Term Arthritis Safety Study (CLASS) study. Reproduced with permission from (26).

events than rofecoxib (mainly myocardial infarctions; relative risk, 0.2). The study authors speculated that naproxen exerts a cardiovascular protective effect similar to aspirin because naproxen is able to reduce TxA_2 synthesis in platelets by 95% and to reduce platelet aggregation by 88% (23, 24). This hypothesis could not be evaluated from the data in the VIGOR trial because the trial did not include a placebo arm, which is typical of clinical trials in arthritis.

The Celecoxib Long-Term Arthritis Safety Study (CLASS) trial compared twice daily doses of 400 mg celecoxib to either twice daily doses of 75 mg diclofenac or thrice daily doses of 800 mg ibuprofen in 8075 patients with osteoarthritis or rheumatoid arthritis (~70% osteoarthritis). The dose of celecoxib was 2 or 4 times higher than the dose recommended for anti-inflammatory activity. Data published after six months of follow-up indicated that celecoxib was associated with a reduced incidence of upper gastrointestinal events compared with the combined NSAID groups (25). Subsequent release of the data obtained from 12 months of study revealed no statistically significant difference in gastrointestinal events between celecoxib, ibuprofen, or diclofenac (**Figure 9**) (26). The latter observation is consistent with the similar COX-2 selectivity of celecoxib and diclofenac as measured by the human whole blood assay. No differences were observed in cardiovascular events between celecoxib, ibuprofen, and diclofenac.

INTRODUCTION TO THE MARKET

The results of the VIGOR and CLASS trials were interpreted to validate the COX-2 hypothesis in human beings; i.e., selective COX-2 inhibitors are anti-inflammatory with reduced gastrointestinal toxicity, although a clear reduction in gastrointestinal toxicity was only observed with rofecoxib. This offered the potential for treatment of debilitating diseases such as rheumatoid arthritis and osteoarthritis to individuals who could not tolerate traditional NSAIDs. Celecoxib was launched six months before rofecoxib and captured sales of approximately $1.5 billion in its first year on the market, making it the most successful drug launch in history. Rofecoxib garnered sales of approximately $400 million in its first year. The initial robust sales of celecoxib and rofecoxib had minimal impact on the sales of traditional NSAIDs. One interpretation of this is that the bulk of

Platelet aggregation: clumping of platelets at the site of vascular injury. Functions to restrict blood loss and provide a surface for fibrin deposition

CLASS: Celecoxib Long-Term Arthritis Safety Study

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celecoxib and rofecoxib sales were to individuals who were unable to take traditional NSAIDs, the target population for selective COX-2 inhibitors.

The launch of celecoxib in February 1999 concluded a remarkable chapter in pharmaceutical history. The COX-2 gene was discovered in 1991, and the protein was expressed and characterized in 1992; drug discovery efforts were launched almost immediately thereafter. The COX-2 hypothesis was validated in animal models in 1994, and five years later selective COX-2 inhibitors were on the market. This is one of the most rapid drug discovery and development efforts for a non-AIDS or noncancer indication and no doubt reflected the long history of clinical experience with traditional NSAIDs, the clear unmet medical need for individuals unable to take NSAIDs because of gastrointestinal sensitivity and the testable hypothesis to meet this need.

Celecoxib and rofecoxib were aggressively marketed from the outset. Rofecoxib advertising expenditures for 2000 were the highest of all marketed drugs and celecoxib ranked seventh (27). Direct-to-consumer advertisements were a major component of both advertising campaigns and these were targeted to the general population, not the subset of NSAID-sensitive patients who would benefit most from them. Hypothetically, this represented a major shift in the risk-benefit equation because the risks were the same for all patients but the benefits were fewer for individuals who could tolerate traditional NSAIDs. The general perception was that these were very safe drugs, so the hypothetical shift in the risk-benefit calculation may have seemed inconsequential. The heavy marketing of COX-2-selective inhibitors contributed to the listing of these drugs on insurance company formularies, which removed price differential as a barrier to adoption of this new class of drugs and dramatically accelerated sales. Combined sales of COXIBs (celecoxib, rofecoxib, valdecoxib) exceeded $5 billion in 2003, and projections for the future were much higher. Postmarketing population-based studies provide varying estimates of the actual benefit of COXIBs relative to NSAIDs in reducing gastrointestinal side effects in patients (28, 29). In fact, chronic use of COXIBs is associated with an increase in gastrointestinal side effects, albeit lower than the increase induced by NSAIDs (28, 29). This may provide clinical support for the finding in rats that the induction of gastrointestinal toxicity is associated more closely with the total extent of reduction of PG biosynthesis than with the identity of the COX enzymes responsible for their synthesis. In fact, a COX-1-selective inhibitor, SC-560, does not induce gastrointestinal lesions in the rat when administered alone but does induce toxicity when coadministered with a COXIB (30).

COX-2 AND CANCER

The importance of COX and prostaglandins in the development and progression of cancer has been recognized since the first report that tumors produce large amounts of PGE_2 (31). Strong preclinical data exist to support multiple mechanisms of COX involvement in tumorigenesis (32). These include carcinogen activation, tumor promotion, resistance to apoptosis, tumor metastasis, and angiogenesis inter alia. The relevance of these various mechanisms to human cancer was somewhat uncertain prior to the discovery in 1991 that individuals who regularly take aspirin exhibit reduced mortality from colon cancer (33). That report, which has been confirmed in many additional epidemiological studies, underscored the importance of PGs in human cancer and suggested a strategy for cancer prevention via COX inhibition. The focus of this strategy narrowed to COX-2 inhibition with the discovery that COX-2 is highly expressed in human colon cancers and benign tumors but not in surrounding normal tissues. The control of COX-2 expression is downstream of epidermal growth factor binding to its receptors, which are major contributors to the growth of many different cancers. As anticipated, COXIBs are powerful inhibitors of tumorigenesis in several animal models. Furthermore, these compounds exhibit potentially important

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adjuvant activity in combination with other antitumor agents (34). This adjuvant activity may be due to the antiangiogenic activity of COXIBs.

Prevention is an attractive strategy for reducing the incidence and mortality of human cancer. In fact, the steady reduction in cancer death rates in the United States over the past decade is largely due to reduction in the incidence of tobacco-related cancers attendant on the sizable reduction in cigarette smoking that has occurred over the past twenty-five years. Prevention with pharmacological agents is an attractive possibility, but one that presents significant practical challenges. The agents must be not only efficacious but also extremely safe because they must be taken for many years (the latency period for carcinogenesis is typically 25–30% of the lifespan of a species in mammals). The safety bar seems too high for prevention trials in the general population because the benefit is uncertain compared with the risk in such a large, heterogenous group. However, the risk-benefit calculation is very different in groups of individuals with a defined, elevated risk of cancer development or in individuals who already have had a primary cancer and are at elevated risk of recurrence.

Colon polyp: a premalignant lesion also called an adenoma that occasionally progresses to colon cancer

APPROVE: Adenomatous Polyp Prevention on Vioxx

APC: Adenoma Prevention with Celecoxib

COLON ADENOMA PREVENTION TRIALS

COXIBs appeared to be ideal cancer preventive agents, especially in individuals at elevated risk (35). They were widely used in the general population and appeared efficacious and safe. Thus, colon polyp recurrence trials were designed in which individuals who had a benign polyp removed following colonoscopy were administered a COXIB for a period of three years. Three parallel trials were conducted with similar designs but different drugs or doses [Adenomatous Polyp Prevention on Vioxx (APPROVE), Adenoma Prevention with Celecoxib (APC) and Prevention of Colorectal Sporadic Adenomatous Polyps (PreSAP)]. In the APPROVE trial, a total of 2586 men and women over forty who had a polyp removed within the previous twelve weeks were enrolled (36). They were randomized to a placebo group or received a daily 25 mg dose of rofecoxib. Exclusion criteria included cardiovascular risk factors such as hypertension and angina. After three years, individuals on rofecoxib exhibited a 24% reduction in the recurrence of polyps compared with those on placebo (37). However, they also exhibited a 1.92-fold increase in the occurrence of cardiovascular side effects from 0.78/100 patient year in the placebo group to 1.50/100 patient year in the rofecoxib group (**Figure 10**). These included myocardial infarctions and ischemic cerebrovascular events. The incidence of cardio- and cerebrovascular events was similar in the placebo and treated groups for the first eighteen months, but then diverged so that by thirty-six months a clear, statistically significant difference existed. Ultimately, 3.6% of the patients in the rofecoxib group developed thrombotic adverse effects compared with 2.0% of the patients in the placebo group.

The APC trial enrolled 2045 patients into three patient groups—a placebo and two doses of celecoxib (200 mg and 400 mg twice a day) (38). After three years, a significant dose-dependent decrease in polyp recurrence was observed. The percentages of patients presenting with an adenoma at three years was 60.7% in the placebo group, 43.2% in the low-dose celecoxib group, and 37.5% in the high-dose group (39). In fact, high-dose celecoxib reduced polyp recurrence in patients with advanced adenomas (i.e., with polyps greater than 1 cm at enrollment) by 67%. However, dose-responsive increases in cardiovascular events also were observed (2.6-fold in the low-dose group and 3.4-fold in the high-dose group, which also correspond to the percentages of patients taking the drug who exhibited thrombotic events compared with patients taking placebo—1.0% thrombotic events) (**Figure 10**).

The PreSAP trial enrolled 1561 patients who were assigned in a 3:2 ratio to celecoxib (400 mg once a day) or placebo (40). Reduction of polyp recurrence was observed at both one and three years for the celecoxib group (36%), and a 51% reduction was observed in the recurrence of

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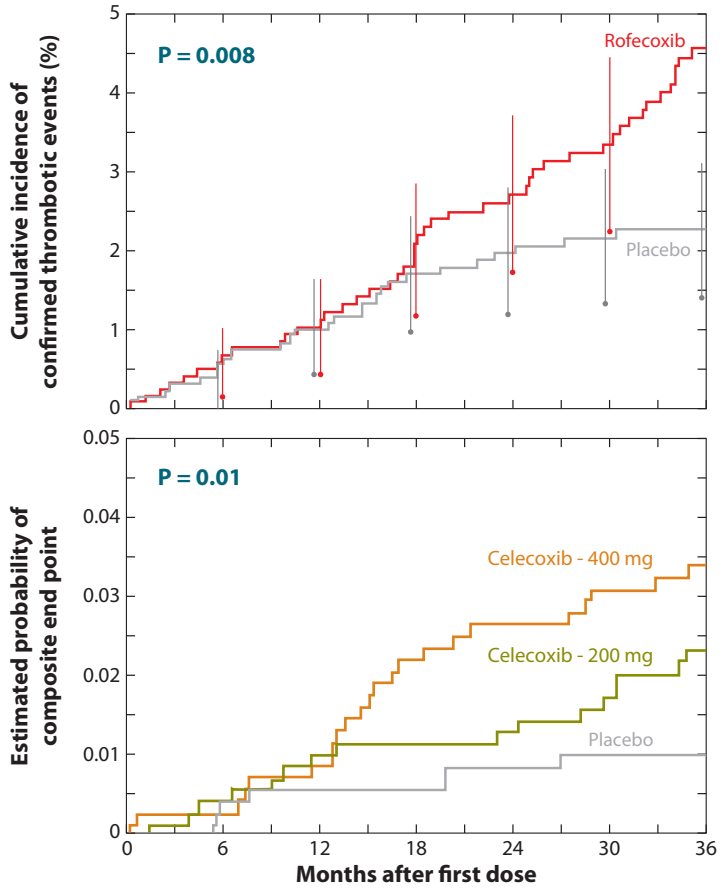

Figure 10

Cumulative incidence of cardiovascular lesions induced by rofecoxib (*left*) and celecoxib (*right*) in the Adenomatous Polyp Prevention on Vioxx (APPROVE) and Adenoma Prevention with Celecoxib (APC) studies. Reproduced with permission from (36, 38).

advanced adenomas. A 1.3-fold increase in cardiovascular side effects was observed in the celecoxib group relative to placebo. These results were consistent with those of the APC trial, albeit of lesser magnitude.

CARDIOVASCULAR EFFECTS

Comparison of the three trials for prevention of polyp recurrence by COX-2 inhibitors validates the hypothesis that these compounds prevent the recurrence (and possibly development) of colon polyps. Chemoprevention is dose responsive, and the reduction in the incidence and size of advanced lesions is greater than that of all lesions. The trials extend to humans the results of preclinical studies, indicating a role for COX-2 in colon carcinogenesis, and validate it as a molecular target for chemoprevention of colonic neoplasia. None of the trials was long enough in duration to evaluate the impact of COX-2 inhibitors on cancer development. However, the trials also establish that cardiovascular effects (myocardial infarctions and strokes) occur with prolonged administration of COX-2 inhibitors. The side effects are also dose responsive and are seen with two structurally

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distinct COX-2 inhibitors. This suggests the cardiovascular side effects are mechanism based and dependent on inhibition of COX-2; i.e., they represent a class effect. This conclusion is supported by the observation of cardiovascular effects in two trials of valdecoxib (with pretreatment with its injectable prodrug, parecoxib) for relief of postsurgical pain in patients undergoing coronary artery bypass grafting surgery and by comparison of the cardiovascular effects of etoricoxib and diclofenac (41–43). Thus, one is confronted with a classic conflict between benefit and risk. In the case of colon cancer prevention, it has been concluded that the risk of cardiovascular side effects negates the benefit of prevention of polyp recurrence (44). This is because the progression of colon cancer is slow, requiring long-term drug use, and because the risk of cancer development is not 100%. The number of cancers that can be prevented does not justify the risk of increased cardiovascular events. The same benefit-risk calculation in a different patient population may yield a different conclusion. For example, celecoxib and the nonselective NSAID sulindac are used for regression of colon polyps in individuals with familial polyposis, an inherited disorder in which patients experience a large tumor burden and a very high rate of progression to cancer.

The impact of the discovery of cardiovascular side effects in the APPROVE trial was swift and dramatic. Merck withdrew Vioxx from the market within days of the report from the Trial's data safety monitoring board. This represented a tremendous financial blow in terms of the loss of continuing sales of the drug (nearly $2.5 billion in 2003) and triggered an immediate avalanche of lawsuits by individuals who had cardiovascular or cerebrovascular events while taking the drug. Given the large number of individuals taking Vioxx, this represented a potentially disastrous liability for the company. In fact, the first trial award of $253 million was stunning, and offered a potentially chilling vision of the future for Merck. The accounts of this trial in the popular press are either amusing or depressing, depending on one's point of view, and underscore the difficulty of adjudicating complex scientific and clinical issues before a lay jury (45). Subsequent trials led to a balanced number of judgments for and against the plaintiffs, but ultimately Merck offered a broad settlement to all plaintiffs. Shortly after the removal of Vioxx, Pfizer withdrew Bextra (valdecoxib) from the market, leaving celecoxib as the only COXIB sold in the United States. Although much of the initial legal focus was on Merck, an increasing number of lawsuits are being filed against Pfizer.

This experience also focused an intense spotlight on the FDA approval process for COXIBs and pharmaceuticals in general. Most of the efficacy and safety trials on which the COXIBs were approved for marketing were relatively short (6–12 months) compared with the three-year time courses of the APPROVE, APC, and PreSAP trials. In fact, no difference in cardiovascular events was observed between placebo and drug-treated groups in the three polyp recurrence trials for up to 12 to 18 months, so it is unlikely that shorter trials would have yielded evidence of cardiovascular side effects. The cardiovascular events reported in the VIGOR trial occurred in individuals treated with a 50 mg dose of rofecoxib, which is higher than the 25 mg anti-inflammatory dose used in the APPROVE trial. Also, there was no placebo control group in VIGOR as is typical in arthritis trials; the elevation in cardiovascular events was relative to a naproxen-treated group. Arguments that naproxen is cardioprotective were advanced to explain the higher cardiovascular events in the Vioxx group.

Cardiovascular disease is the most common cause of death in the United States; thus, in the absence of long-term, placebo-controlled safety studies such as those embedded in the APPROVE, APC, and PreSAP trials, it is unlikely that the adverse effects of chronic use of COXIBs would have been uncovered even in large-scale postmarketing surveillance. This is a major concern for the safety evaluation of all new pharmaceuticals. The low incidence of cardiovascular events recorded in the polyp prevention studies means that trials designed to probe for such side effects may need to be large, long, and expensive.

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MECHANISMS OF CARDIOVASCULAR SIDE EFFECTS

As mentioned above, there are strong clinical data indicating that the cardiovascular side effects are mechanism based; i.e., they result from the inhibition of COX-2. There are also strong data to suggest that this is due to the reduction of the biosynthesis of PGI_2 and perhaps PGE_2, to some extent, in vessel wall (**Figure 11**). The reduction in PGI_2 biosynthesis in individuals treated with COX-2 inhibitors was established by FitzGerald and colleagues coincident with the introduction of celecoxib and rofecoxib (46). This was an unprecedented finding at the time because prior preclinical data showed COX-1 as the major cyclooxygenase in blood vessels. Despite some initial skepticism that the drop in PGI_2 levels in individuals taking COXIBs reflected biosynthesis in the vessel wall, the clinical observation is consistent with results demonstrating that COX-2 is induced in cultured vascular endothelial cells exposed to laminar flow stress (47).

PGI_2 is a potent vasodilator and reduces the responsiveness of platelets to proaggregatory substances (48). It also reduces atherogenesis in hyperlipidemic mice, so it may act to reduce vascular inflammation, thrombosis, and atherosclerosis by acute or chronic mechanisms (49). Obviously, reducing its biosynthesis by the administration of COXIBs represents a risk factor for cardiovascular disease. However, an intriguing and important question is why the magnitude of the cardiovascular events required more than 12–18 months to achieve a statistically significant difference from placebo. One component to a possible answer is that it is related to the magnitude of the cardiovascular challenge. The groups in the polyp prevention trials were generally healthy, and individuals with cardiovascular disease were excluded. In fact, as mentioned above, individuals in two coronary artery bypass grafting trials administered valdecoxib for postsurgical pain exhibited a significant increase in myocardial infarctions and strokes in 10 to 14 days. However, it also may be possible that the lengthy delay in the appearance of cardiovascular side effects in the polyp prevention trials is a consequence of chronic reduction in PGI_2 biosynthesis, which may predispose to atherosclerosis or rupture of an atherosclerotic plaque.

A related question is why the percentages of individuals who exhibited cardiovascular adverse effects is so low (2–3% of the total number of patients taking COXIBs after three years). Although there is considerable individual variation in the magnitude of the inhibitory response to COX-2 inhibitors (50), most of the individuals on trial were experiencing some reduction in PGI_2

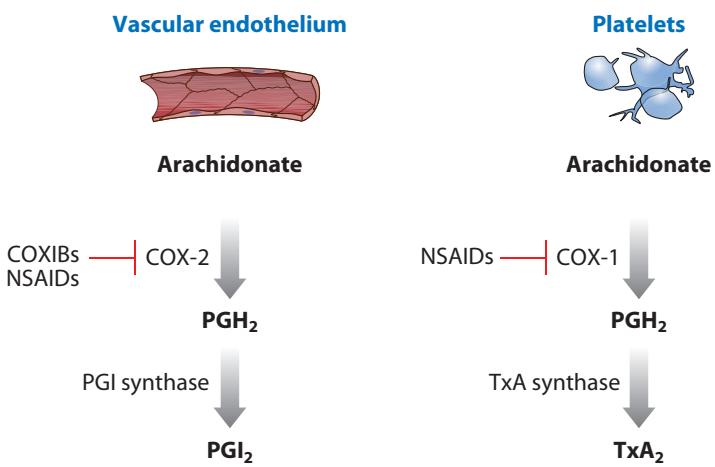

Figure 11

Oxygenation of arachidonic acid to PGI_2 in vascular endothelial cells and TxA_2 in platelets.

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biosynthesis. In fact, the data for polyp recurrence reduction in a high-dose celecoxib group implies nearly 70% of the individuals experienced a biologically significant reduction in PG production in the colon. Although the answer to the low-incidence question may be another reflection of the low cardiovascular hazard of these patients, other factors may be operative. For example, Anning et al. have reported that COX inhibitors do not elevate blood pressure or increase vascular contractility in mice unless the animals are pretreated with agents that reduce nitric oxide biosynthesis (51). The authors propose that nitric oxide is the primary mediator of vascular reactivity, and the importance of PGI_2 is only revealed when nitric oxide levels are reduced. This suggests that individuals with conditions that reduce vascular nitric oxide biosynthesis (e.g., diabetes, smoking) would be particularly prone to the effect of COXIBs. Indeed, the majority of cardiovascular events in the APC trial (celecoxib) occurred in individuals with risk factors for cardiovascular disease.

Endocannabinoid: endogenous ligand for a cannabinoid receptor (CB1 or CB2)

The complexity of the interplay of PGs and other mediators of vascular biology has been detailed in an insightful review by Grosser et al. (52). A recent twist on the PGI_2 mechanism is worth noting. COX-1 and COX-2 oxygenate arachidonic acid to PGG_2 at comparable rates. However, COX-2 carries out the selective oxygenation of endocannabinoids, i.e., 2-arachidonoylglycerol and 2-arachidonoylethanolamide, to PGG_2 glycerol ester and ethanolamide, respectively, which are converted to prostaglandin derivatives. Ghosh et al. recently reported that the glyceryl ester of PGI_2 (i.e., PGI_2-G) is an agonist for $PPAR_\delta$ (53). In vascular endothelial cells, the activation of PPARδ represses the synthesis of the prothrombotic tissue factor and protects against thrombotic disorders (**Figure 12**). Reduction of PGI_2-G synthesis by a COX-2 inhibitor enhances the synthesis and release of tissue factor, thereby increasing the probability of cardiovascular events.

A critical and incompletely resolved question is whether COX-2 selectivity matters from the standpoint of adverse cardiovascular effects. All NSAIDs and COXIBs, whether they are nonselective or selective, inhibit COX-2. Does any compound that inhibits the biosynthesis of PGI_2 or PGI_2-G in the vessel wall increase the risk of cardiovascular disease regardless of whether it inhibits COX-1? Non-selective (i.e., COX-1/COX-2) inhibitors decrease the production of TxA_2 in platelets, which may offset the prothrombotic effects of inhibition of PGI_2 in vascular endothelial cells and reduce cardiovascular risk (52). However, most NSAIDs other than aspirin and naproxen do not inhibit COX-1 in platelets strongly enough to depress TxA_2 synthesis for a prolonged period of time.

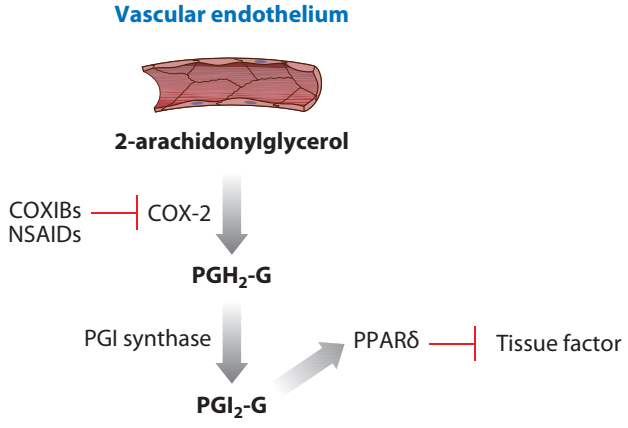

Figure 12

Oxygenation of 2-arachidonoylglycerol by COX-2 in vascular endothelium to PGI_2-glyceryl ester and control of tissue factor expression by PPARδ.

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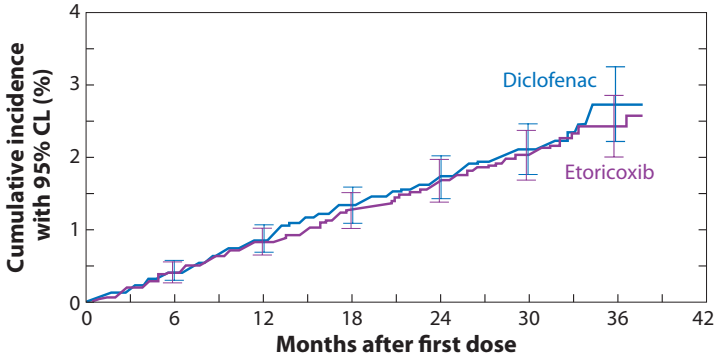

Figure 13

Cumulative incidence of myocardial infarction, stroke, or vascular death in the MEDAL study comparing diclofenac (*blue*) and etoricoxib (*red*). Reproduced with permission from (43).

Limited clinical data are available to inform the issue of whether nonselective NSAIDs induce adverse cardiovascular effects. Long-term clinical studies analogous to APPROVE, APC, and PreSAP have not been conducted with nonselective NSAIDs. The best clinical data have been generated in studies where NSAIDs were compared with COX-2 inhibitors for gastrointestinal side effects. However, these studies were of short duration, usually less than one year, which according to the analysis of the polyp prevention studies described above, is insufficient to observe a statistically significant increase of cardiovascular events. Nevertheless, meta-analysis of these studies suggests that traditional NSAIDS such as ibuprofen, indomethacin, and diclofenac are associated with an increased risk of cardiovascular side effects (54). This increased risk has been confirmed for diclofenac by a long-term study comparing diclofenac and etoricoxib (**Figure 13**). As discussed earlier, diclofenac exhibits higher COX-2 selectivity in vivo than anticipated from its activity against purified proteins, and is equivalent to celecoxib in that regard (43). Thus, diclofenac should probably be considered a COX-2-selective inhibitor from the standpoint of cardiovascular risk and, in fact, gastrointestinal toxicity. Interestingly, although diclofenac exhibits comparable gastrointestinal toxicity to celecoxib, it exhibits higher gastrointestinal toxicity in patients than etoricoxib, implying that etoricoxib is a more selective COX-2 inhibitor in the gastrointestinal tract than either diclofenac or celecoxib. Curiously, in the meta-analysis of cardiovascular risk data, naproxen appears to be associated with a reduced risk relative to COXIBs and other NSAIDs (54). Whether this difference is real is uncertain, but chronic naproxen does suppress platelet TxA_2 synthesis more than most other NSAIDs (24).

Because low-dose aspirin is an effective prophylactic agent against cardiovascular disease, one strategy for negating the side effects associated with COXIBs is to combine them with low-dose aspirin. Epidemiological analysis of coadministration of aspirin and a COXIB is associated with an incidence of gastrointestinal side effects comparable to those of the comparator NSAID, which eliminates the justification for the use of the COXIB (28). The ability of low-dose aspirin to increase the gastrointestinal toxicity of COXIBs appears to be due to its inhibition of COX-1 in blood platelets (18). The effect of low-dose aspirin on PG synthesis in most tissues is transient, but the effect on platelets is prolonged for the lifetime of the platelet. Aspirin irreversibly inactivates COX enzymes, and because platelets lack nuclei they are unable to replace the inactivated COX-1 that triggers the biosynthesis of the proaggregatory, TxA_2 (55). This cellular selectivity is the molecular basis for the cardiovascular protective activity of aspirin because TxA_2 contributes to acute

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thrombotic disorders as well as to chronic proatherogenic events. Inhibition of platelet aggregation and the consequent increase in bleeding is also a component of gastrointestinal toxicity (18).

WHAT HAVE WE LEARNED?

The past twenty years in the history of NSAIDs have been a wild ride. The discovery of COX-2 generated tremendous excitement, insight into the etiology of multiple chronic diseases, and hope that those diseases could be treated more effectively, or at least more safely. It also ignited a world-class race to bring the first COXIB to market—a race that showcased all the modern tools of medicinal chemistry, drug discovery, and molecular pharmacology. The success in bringing celecoxib and rofecoxib to market in such a short time underscores the power of integrating these complementary disciplines in an intense and focused manner. Given the long history of human use of NSAIDs, the rationale for the clinical development of COXIBs seemed to be straightforward. But the original COX-2 hypothesis was oversimplified and ignored the possibility that expression of COX-2 exerts beneficial as well as harmful effects. So in spite of enormous excitement, hard work, and massive investment, the net result is that there is one additional NSAID/COXIB on the market in the United States (celecoxib) and two in Europe [celecoxib and etoricoxib (lumiracoxib is being withdrawn from several markets for liver toxicity unrelated to COX-2 inhibition)]. There is also a realization that some traditional NSAIDs (e.g., diclofenac, meloxicam) have reasonable COX-2 selectivity in vivo.

Many drugs are marketed directly to consumers through mass media outlets. In the case of COXIBs, marketing campaigns expanded drug sales from NSAID-intolerant individuals for whom they were developed to the general population. This expansion of the patient profile altered the risk-benefit equation for the use of COXIBs. The increase in sales was aided and abetted by reimbursement policies that removed price barriers to new drug adoption. All of this reflects the blockbuster model of drug development that large pharmaceutical companies have adopted. Given the costs of bringing new drugs to market and the demands of shareholders for steadily increasing stock prices, it is easy to understand the appeal of this model. Furthermore, the health care system depends heavily on the pharmaceutical industry to hold total costs down by keeping people out of hospitals. These pressures will increase as people live longer. But the heavy dependence of the financial health of companies on outsized sales of a few products exposes them to severe risk if anything goes wrong with a drug at any stage of its clinical lifetime. In the case of Vioxx, we have witnessed the severe penalties exacted by the financial markets and the legal system when something does go wrong.

FUTURE PERSPECTIVE

The COXIB experience underscores the critical balance between efficacy and safety, between benefit and risk. The emphasis of clinical perspectives and articles in the lay press over the past four years has been on safety and the design of trials to measure it. Ironically, the way forward for COXIBs and related NSAIDs may depend on efficacy. For example, etoricoxib is a very potent and selective COX-2 inhibitor in vitro and exhibits selectivity in the human whole blood assay second only to lumiracoxib. A long-term safety study showed it has very similar cardiovascular toxicity to diclofenac, leading to the claim by its manufacturer that it is as safe as an NSAID (see above, however, regarding the classification of diclofenac) (43). Using these and other data, Merck petitioned the FDA in 2007 to market etoricoxib for a range of indications, including arthritis, pain, gout, and ankylosing spondilitis. The FDA advisory panel voted overwhelmingly to deny the petition on the basis that etoricoxib was no more effective as an anti-inflammatory

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agent than other COXIBs or NSAIDs on the market. One might contend that this decision is inconsistent with the emerging epoch of personalized medicine, when one should have more keys on the clinical key-ring to treat chronic diseases. However, given the cardiovascular toxicity of COXIBs, the uncertainty of whether NSAIDs exert such effects, and the lack of clinical trial data showing COXIBs are superior to NSAIDs as anti-inflammatory or analgesic agents, it is easy to understand the FDA's decision.

In an attempt to determine where COXIBs could be efficacious and where their benefit might justify their risk, one should probably look to situations where COX-1 inhibition is deleterious. COXIBs were developed to service individuals with extreme sensitivity to the gastrointestinal effects of nonselective NSAIDs, and this is still an important indication. Another is prevention or treatment of cancer, likely in the adjuvant setting. Combination treatments are standard of care for most cancers, and given the importance of COX-2 in the etiology of multiple cancers, it seems likely that COXIBs will find a niche in cancer treatment. COX-2-selective inhibitors should be preferable to NSAIDs in cancer treatment or prevention because of the sensitivity of cancer patients to bleeding disorders, which are exacerbated by inhibition of COX-1 in the platelet.

The impressive efficacy of celecoxib as a single agent in the prevention of colon polyp recurrence was extended in a recent study combining the ornithine decarboxylase inhibitor, difluoromethylornithine (DFMO), and the NSAID, sulindac (56). DFMO inhibits carcinogenesis in animal models and sulindac induces polyp regression in familial polyposis patients, a group at high risk for development of colorectal cancer. Prior to initiating the combination trial, a dose de-escalation study was performed to define the lowest dose of DFMO that inhibits polyamine biosynthesis in colon mucosa. This was determined to be 500 mg. The dose chosen for sulindac was 150 mg, which is half the normal anti-inflammatory dose. The design of the prevention trial was similar to those of the APPROVE or APC trials in that patients from whom polyps had been removed were randomized to a placebo group or a DFMO/sulindac group. After three years, the DFMO/sulindac combination reduced recurrence of all polyps by 70% and advanced adenomas by 92%. This is an exciting result that may form the basis for a larger study. The small size of the current study did not allow conclusions to be made about potential cardiovascular effects of the low dose of sulindac. Sulindac also is of interest because it or its metabolites exert several non-COX-related effects, some of which may contribute to its ability to reduce the growth of neoplastic cells in the colon (57). It will be interesting to probe the role of these off-target effects in polyp reduction.

If nothing else, the COXIB experience has taught us that COX-2 is important in normal physiology as well as acute and chronic diseases. We have learned a great deal about the mechanism of COX-2 control and the contribution of the PGs it synthesizes to a broad range of clinical conditions. The challenge is to use this information either by targeting COX-2 directly or altering the arachidonic acid cascade downstream of it to reduce PG tone in settings where it is excessive, and to do this in a way that minimizes risks to the individual. Given the long history of NSAIDs, the past twenty years represent a tumultuous but brief period that has taught us valuable lessons and raised significant challenges. Tackling these challenges will require integration of the tools of drug discovery and molecular medicine with clinical advances based on emerging concepts of individual benefit and risk.

SUMMARY POINTS

1. The discovery of COX-2 was believed to provide a molecular target for compounds with anti-inflammatory and analgesic activity but reduced gastrointestinal toxicity (the COX-2 hypothesis).

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2. The COX-2 hypothesis assumed that COX-2 plays a role in pathophysiology but not in normal physiology. The hypothesis also assumed that all prostaglandins biosynthesized in the gastrointestinal tract that are cytoprotective are derived from COX-1. Both assumptions proved to be oversimplified.
3. Outstanding efforts in medicinal chemistry led to the rapid discovery, optimization, and development of COXIBs.
4. COXIBs exhibit anti-inflammatory activity comparable to traditional NSAIDs but reduced gastrointestinal toxicity. Coadministration of low-dose aspirin significantly reduces the differential in gastrointestinal toxicity between COXIBs and NSAIDs.
5. COXIBs and certain NSAIDs reduce the recurrence of colon polyps in patients. The percentage reduction is greatest in individuals with advanced adenomas.
6. An unanticipated discovery of the colon polyp recurrence clinical trials was that COXIBs induce cardiovascular toxicity in a mechanism-based fashion. This appears to be because of their ability to inhibit the COX-2-dependent biosynthesis of PGI_2 in vascular endothelium.
7. Epidemiological data suggest that certain NSAIDs induce cardiovascular toxicity comparable in magnitude to COXIBs, but the data are from studies that are too short in duration to have high statistical significance. The cardiovascular toxicity of NSAIDs remains to be definitively evaluated.
8. The COXIB experience provides a classic example of the balance of risk and benefit and illustrates the complexities of the development, evaluation, and marketing of new drugs.

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DISCLOSURE STATEMENT

The author holds several patents for COX-2 inhibitors.

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