

MINIREVIEW

Cox-2-Selective Inhibitors: The New Super Aspirins

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Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin or ibuprofen are commonly used for the occasional headache or fever or to reduce soreness and inflammation resulting from work or exercise. However, many individuals who have rheumatoid arthritis and osteoarthritis depend on these drugs to reduce pain and to restore sufficient flexibility in inflamed joints to permit normal day-to-day activities. Until now, the beneficial effects of NSAIDs have come with a price; about 1% of chronic users per year develop ulcers or other serious gastrointestinal complications (Chase, 1998). Because of the widespread use of NSAIDs, these toxicities are one of the most prevalent drug-associated health risks.

In December 1998, the first in a new family of cyclooxygenase-2 isozyme (Cox-2) inhibitors, celecoxib (SC58635; Celebrex), was approved. These second-generation NSAIDs, which preferentially inhibit Cox-2, promise to have the same anti-inflammatory, antipyretic, and analgesic activities as current NSAIDs. However, unlike present-day NSAIDs, which also inhibit prostaglandin synthesis by Cox-1 in the stomach lining, Cox-2-selective inhibitors are not expected to cause the gastrointestinal complications that last year hospitalized an estimated 76,000 NSAID users in the United States. In an NSAID market that amounted to \$1.9 billion in prescription sales in America in 1997, estimates from Wall Street are that worldwide sales of the first Cox-2 inhibitors could eventually reach \$1 to \$3 billion annually (Chase, 1998).

Rationale for Cox-2-Selective Drugs

The development of the latest generation of NSAIDs began with the unexpected discovery, in 1991, of a second cyclooxygenase isozyme. Two groups of researchers, one studying genes elevated in transformed chicken fibroblasts (Simmons

et al., 1989) and another studying genes induced by phorbol esters in murine fibroblasts (Kujubu et al., 1991), independently discovered a second cyclooxygenase gene that appeared, based on its pattern of regulation and expression, to be the sole isozyme that produced prostaglandins responsible for potentiating inflammatory processes (for a review, see Smith et al., 1996). The realizations that inhibition of Cox-2 might be sufficient to achieve the therapeutic benefits of NSAID therapy and, conversely, that the indiscriminate inhibition of Cox-1 likely resulted in the side effects commonly associated with NSAIDs stimulated an intense and highly competitive race to identify compounds that would selectively inhibit only Cox-2.

Development of Cox-2-Selective Inhibitors

The first Cox-2-selective compounds to be identified in these searches were DuP697 (Gans et al., 1990) and NS-398 (Futaki et al., 1993) (Fig. 1), two NSAIDs already in development when Cox-2 was discovered. These compounds had previously been singled out for their gastrointestinal sparing properties in animal models, and when tested using recombinant human cyclooxygenases (Meade et al., 1993; Barnett et al., 1994; O'Neill et al., 1994; Kargman et al., 1996b; Riendeau et al., 1997), they were shown to be 80- and 1000-fold selective, respectively, for inhibition of Cox-2 (Gierse et al., 1995). Ironically, although the development of NS-398 and DuP697 was later discontinued, the structure of DuP697 served as a starting point for the synthesis of the diarylhetereocyclic family of selective inhibitors, which include SC58635 (celecoxib) and MK-966 (rofecoxib) (Seibert et al., 1994; Penning et al., 1997; Prasit and Riendeau, 1997) (Fig. 1).

Depending on the in vitro assay system used for screening, selective drugs were found to be 10 to several thousand times better inhibitors of Cox-2 than of Cox-1 in in vitro assays (Barnett et al., 1994; O'Neill et al., 1994; Gierse et al., 1995; Kargman et al., 1996b; Riendeau et al., 1997). More importantly, all selective inhibitors reacted in a fundamentally

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¹ For clarity, I used the numbering system starting with methionine 1 of the ovine Cox-1 enzyme. The homologous amino acids corresponding to Cox-1 and Cox-2 that are specifically discussed in the text are (Cox-1/Cox-2) Arg¹²⁰/Arg¹⁰⁴, Iso⁴³⁴/Val⁴²⁰, Phe⁵¹⁸/Phe⁵⁰⁴, Iso⁵²³/Val⁵⁰⁹, His⁵²³/Arg⁵¹⁹, and Tyr³⁸⁵/Tyr³⁷¹.

ABBREVIATIONS: Cox-1, cyclooxygenase-1; Cox-2, cyclooxygenase-2; NSAID, nonsteroidal anti-inflammatory drug.

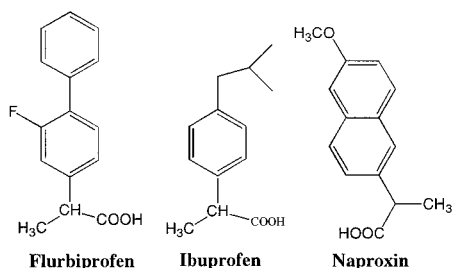
different way with Cox-2 than with Cox-1 (Copeland et al., 1994). Cox-2 inhibitors formed tight binding complexes with this isozyme that dissociated only slowly, whereas if they inhibited Cox-1 at all, they did so in a competitive and freely reversible manner (Fig. 2). This tight-binding mechanism is referred to as time dependent because full inhibition is achieved only on incubation with inhibitor. Many nonselective NSAIDs are time-dependent inhibitors of both Cox-1 and Cox-2 (Rome and Lands, 1975). However, the selective Cox-2 drugs identified by the in vitro tests were all time-dependent inhibitors of Cox-2 but simple competitive inhibitors of Cox-1. These mechanistic findings provided the first qualitative parameter with which to confirm Cox-2 selectivity.

When the search for Cox-2 inhibitors began, the role of Cox-2 in inflammation was simply a hypothesis, and little was known about the relative roles of Cox-1 and Cox-2 in fever and pain. An anti-inflammatory agent without analgesic properties would have been without practical value. Fortunately, it was quickly demonstrated that Cox-2-selective drugs were as effective as nonselective NSAIDs in animal models of inflammation and, as predicted, did not cause ulcers or detectable gastrointestinal bleeding, even at con-

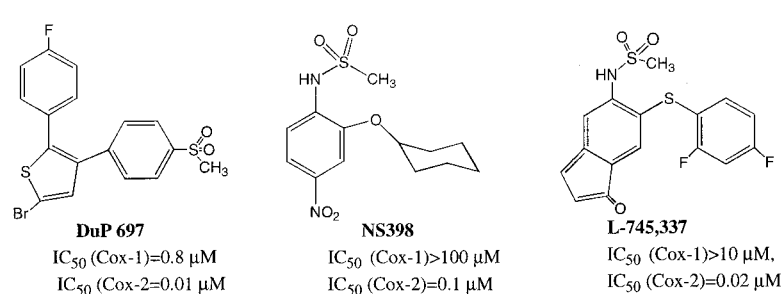
centrations well above their anti-inflammatory doses (Gans et al., 1990; Masferrer et al., 1994; Seibert et al., 1994). Importantly, these compounds were also shown to possess potent analgesic and antipyretic activities (Masferrer et al., 1994; Chan et al., 1995). These and other in vivo studies carried out with rat, dog, and nonhuman primates, together with more recent clinical studies, have led to general acceptance of the Cox-2-inflammation paradigm.

At least seven different major structural classes of Cox-2-selective inhibitors have been identified, including the diarylheterocyclics (or tricyclics), acidic sulfonamides, and 2,6-ditert-butyl phenols, as well as the derivatives of the nonselective inhibitors zomepirac, indomethacin, piroxicam, and aspirin (Fig. 1) (see Prasit and Riendeau, 1997, and Marnett and Kalgutkar, 1998, for references relating to the synthesis and evaluation of these compounds). The most diverse class of these inhibitors, and the first to be approved for human use, consists of diarylheterocyclic compounds related to DuP697 (Fig. 1). Members of this class of molecules possess a basic structural motif in which a *cis*-stilbene framework is fused with a variety of heterocyclic and carbocyclic rings. Although the nature of the ring structure bridging the

Common Non-Selective NSAIDs



Prototypical Cox-2 Inhibitors



Diarylheterocyclic Cox-2 Inhibitors

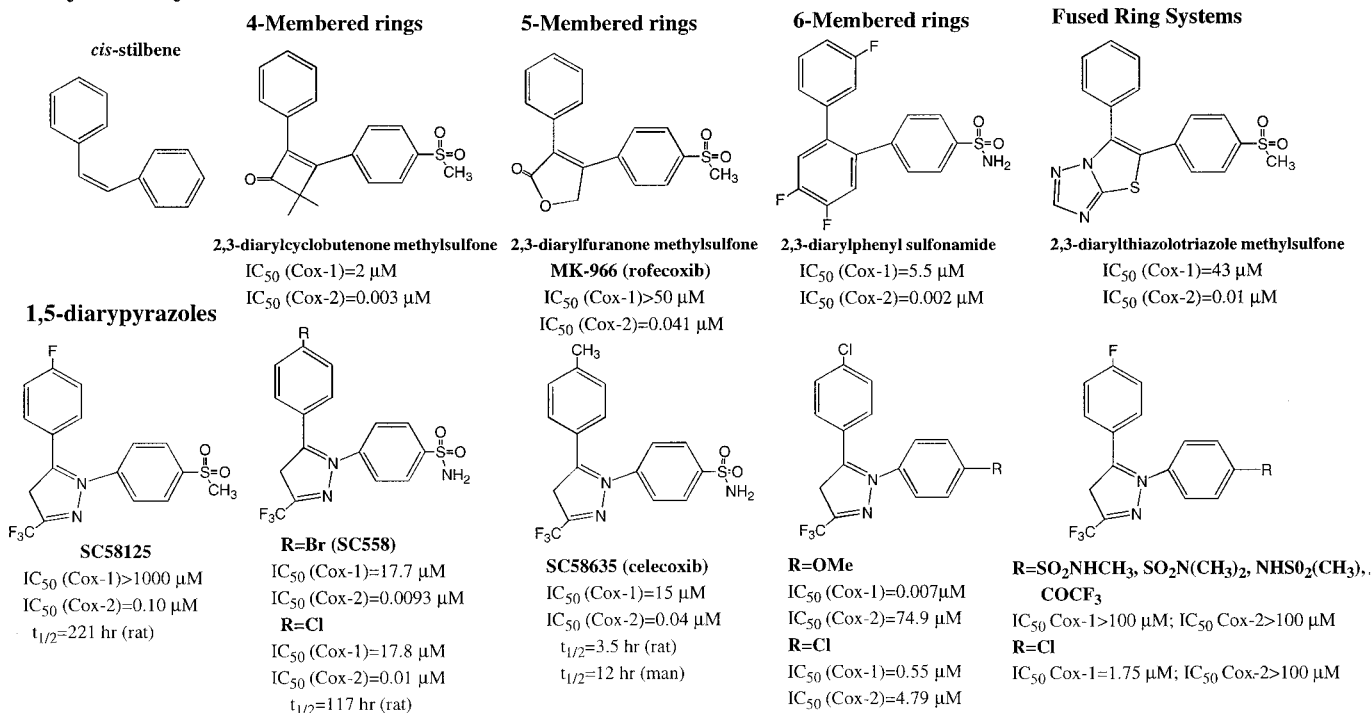


Fig. 1. Structures of representative nonselective and Cox-2-selective NSAIDs.

phenyl constituents does not seem to be particularly critical, the presence of a 4-methylsulfone or 4-methylsulfonamide substituent on one of the phenyl rings that compose the stilbene framework is absolutely essential (Leblanc et al., 1995; Penning et al., 1997). An examination of the structure-activity relationship studies carried out during the development of celecoxib (SC58635) (Penning et al., 1997), the first Cox-2 approved for human use, illustrates some of the structural features that are important for isozyme selectivity within this class of compounds and provides insight into the decision making involved in selecting a drug for clinical trials.

Searle developed celecoxib (SC58635; Celebrex) from the 1,5-diarylpyrazoles, a class of compounds in which the *cis*-stilbene framework is fused to a pyrazole ring (Fig. 1) The activity of these compounds, like that of other diarylheterocyclic inhibitors, is absolutely dependent on either a 4-methylsulfonylphenyl or 4-sulfonamoylphenyl substituent on the pyrazole ring. Substitution of the methylsulfonyl or sulfonamido groups with *N,N*-dimethylsulfonamido, methanesulfonamido, nitro, or trifluoroacetyl moieties abolishes both Cox-1 or Cox-2 inhibitory activity, whereas substitution with a chlorine or a methoxy group produces potent and selective inhibitors of Cox-1 (Leblanc et al., 1995; Penning et al., 1997) (Fig. 1). The limited number of chemical substituents at this position that result in a selective Cox-2 inhibitor suggests that the 4-methylsulfonylphenyl and 4-sulfonamoylphenyl groups interact with specific residues within the Cox-2 NSAID pocket, a prediction that is corroborated by crystallographic structures (see below) (Fig. 3D) (Kurumbail et al., 1996).

In vitro assays with the 1,5-diarylpyrazoles (Penning et al., 1997) and other classes of diarylheterocyclics (Leblanc et al., 1995) showed that the methylsulfone derivatives (e.g., SC58125; Fig. 1) were generally more potent inhibitors of Cox-2 and that these compounds had the highest selectivity (>1000) [selectivity is defined as the ratio of IC₅₀ values for Cox-1 and Cox-2: IC₅₀(Cox-1)/IC₅₀(Cox-2)]. Nevertheless, the

sulfonamide inhibitors (e.g., SC58635) had superior in vivo activity (bioavailability), especially in a rat model of arthritis (Penning et al., 1997). In the end, the medium selectivity (≈300) and high in vivo activity obtained with the sulfonamide compounds were chosen by Searle to be the most favorable combination of characteristics for its first Cox-2 inhibitor.

A final consideration in these structure-activity relationship studies was identifying a drug with favorable metabolic properties. Although many of the 1,5-diarylpyrazoles that possessed 5(4-halophenyl) substituents (e.g., SC58128 and SC588; Fig. 1) had good selectivity and in vivo activity, they also possessed unacceptably long in vivo half-lives (>100 h) (Penning et al., 1997). Substitution of the 5(4-halophenyl) substituents on the pyrazole ring of these compounds with either methoxyphenyl or with methylphenyl, as in SC58635, reduced the half-life to a pharmacokinetically manageable 3 to 6 h in rats and about 12 h in humans (Penning et al., 1997; Prasit and Riendeau, 1997).

Endoscopy studies comparing celecoxib with placebo have also detected no differences in stomach irritation with this NSAID, even at concentrations 3 to 4 times above the anti-inflammatory dose (Lipsky and Isakson, 1997). Both celecoxib and the Merck inhibitor MK-966 have been found to be effective analgesic agents in models of dental pain (Lane, 1997) and effective anti-inflammatory and analgesic agents in patients with rheumatoid arthritis and osteoarthritis (Ehrich et al., 1997; Lane, 1997; Lipsky and Isakson, 1997; Zhao et al., 1997).

It will be interesting to compare the clinical characteristics of celecoxib with Merck's lead compound, MK-966 (Vioxx), which is in stage III clinical trials and will likely be approved in early 1999. MK-966, a methylsulfone derivative, has a longer half-life and is slightly more potent and selective in vitro than the sulfonamide celecoxib. If the increased in vitro potency and selectivity of MK-966 translate to an increased in vivo potency and selectivity, this could improve the safety or efficacy of this NSAID.

Mechanisms of NSAID Inhibition of Cox-1 and Cox-2

Both Cox-1 and Cox-2 catalyze the two-step conversion of arachidonate to prostaglandin H₂, the common intermediate in all prostaglandin synthesis (for a comprehensive review, see Smith et al., 1996). The first step of this process takes place in the cyclooxygenase active site when two molecules of oxygen are added to arachidonate to form the bicyclic peroxide intermediate, prostaglandin G₂. This intermediate must next diffuse to the peroxidase active site, located on the opposite side of the enzyme, where it is reduced to prostaglandin H₂. All NSAIDs bind only in the cyclooxygenase active site and do not affect peroxidase activity.

The cyclooxygenases have an unusual and unique orientation within the membrane (Fig. 3A). Although these enzymes are integral membrane proteins, they do not contain trans-membrane sequences. Four amphipathic helices form a hydrophobic surface that floats or anchors these enzymes in what can be described as an upright position on the membrane. These amphipathic helices form the base of the molecule, and they also form the opening to the cyclooxygenase active site, a hydrophobic pocket that projects inward from the membrane surface of the enzyme. As these helices are buried within the membrane, fatty acid substrates and

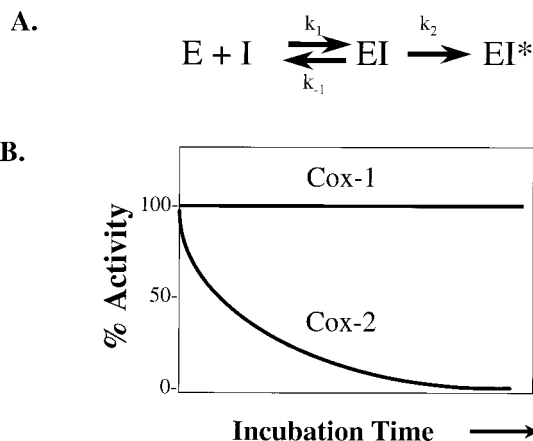


Fig. 2. A, simple competitive inhibitors of the cyclooxygenase form only freely reversible complexes (EI) with the enzyme. Time-dependent inhibitor can in addition form tightly bound slowly reversible complexes (EI*). This process is poorly understood but is thought to involve NSAID-induced conformational changes after the binding of NSAIDs in the cyclooxygenase active site. B, all Cox-2-selective NSAIDs are time-dependent inhibitors of Cox-2 but only simple competitive inhibitors of Cox-1. Thus, at the appropriate concentrations, incubation with Cox-2-selective inhibitors can lead to a gradual and near-complete inhibition of Cox-2 without significantly affecting Cox-1 activity.

NSAIDs must pass through the lipid bilayer to reach the entrance to the cyclooxygenase active site.

Inhibition by Nonselective Cox-1 and Cox-2 NSAIDs. To understand the mechanism for selectivity of Cox-2 inhib-

itors, it is useful to first examine how arachidonate and nonselective NSAIDs bind within the active sites of Cox-1 and Cox-2 (Fig. 3). One of the few charged amino acids in the Cox-1 active site is Arg¹²⁰.¹ Crystal structures of the mouse

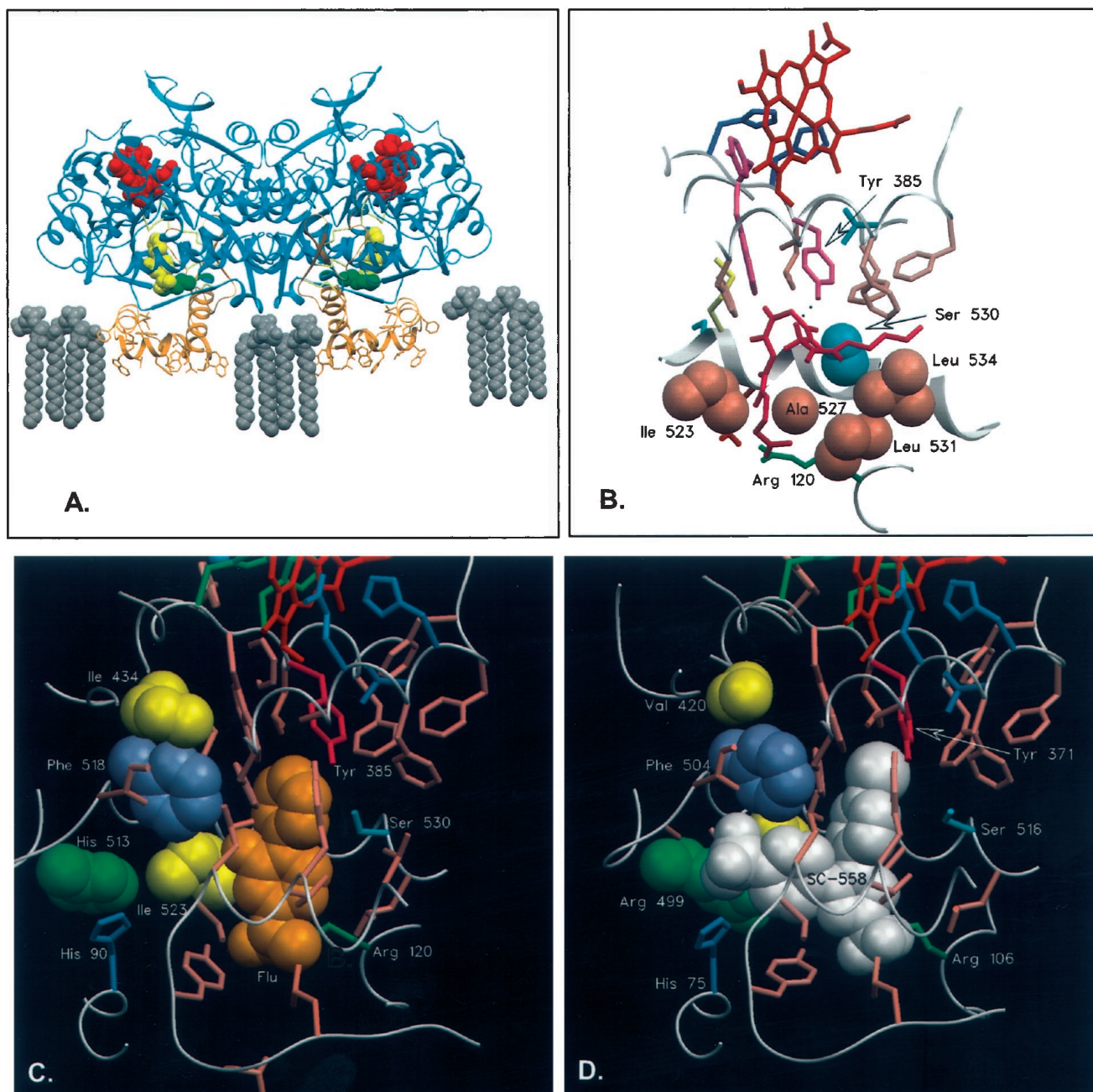


Fig. 3. A, ribbon diagram of the ovine Cox-1 dimer (Picot et al., 1994). Both Cox-1 and Cox-2 have been localized to the luminal surface of the endoplasmic reticular and nuclear membranes, where they anchored into a single leaflet of the lipid bilayer by a membrane binding domain (gold) composed of four amphipathic helices. The main catalytic domain is in blue. Arg¹²⁰ (green) is seen complexed with flubiprofen (yellow), and the heme is shown in red. B, hypothetical orientation of arachidonate within the substrate binding pocket of ovine Cox-1 modeled after the structure determined for the murine Cox-2 apoenzyme complexed arachidonate (R. Kurumbail, Second International Workshop on Cox-2, July 28–31, 1998). Binding of arachidonate within the Cox-1 active site is dependent on coordination with Arg¹²⁰ (green). Abstraction of hydrogen from carbon 13 by the radical form of Tyr³⁸⁵ initiates the cyclooxygenase reaction. Ser⁵³⁰ (blue), near carbon 15, is the site of acetylation by aspirin. C, cut-away view of flubiprofen (Flu) (gold) bound in the hydrophobic channel of Cox-1 (Picot et al., 1994). Critical amino acids that contribute to differential NSAID binding are shown as space-filling molecules. D, cut-away view of SC558 (white) complexed with murine Cox-2. (Coordinates used to prepare this figure were obtained from PDB entry; ICX2, R. Kurumbail, et al. Numbering of amino acid residues is for the murine Cox-2.) Ile⁵²³ (yellow) in Cox-1 (Fig. 3C) is replaced by Val⁵⁰⁹ in Cox-2. The view of Val⁵⁰⁹ is obscured by SC558, which in Cox-2 is able to fit into a side pocket made accessible by the smaller side chain of this amino acid. The substitution of Ile⁴³⁴ (yellow) in Cox-1 (Fig. 3C) for the smaller valine at the homologous position in Cox-2 (Val⁴²⁰, yellow) increases the mobility of Phe⁵⁰⁴ (blue), allowing this amino acid to swing out of the way, further increasing access of the sulfonamide moiety of SC558 to this side pocket. Interaction of Arg⁴⁹⁹ (green) in this side chamber with the sulfonamide or methylsulfoxide groups of SC558 and other diarylheterocyclic Cox-2-selective NSAIDs is thought to be necessary for the tight binding that results in time-dependent inhibition.

apo-Cox-2 enzyme complexed with arachidonate indicate that the carboxylic moiety of this fatty acid forms a salt bond with the guanidinium group of Arg¹²⁰ (R. Kurubail, Second International Workshop on Cox-2, July 28–31, 1998). From Arg¹²⁰, near the mouth of the hydrophobic pocket, arachidonate snakes its way up into the active site, eventually forming a hairpin turn between carbons 9 and 11 and returning back down the hydrophobic channel (Fig. 3B), orientating arachidonate so that the addition of two molecules of dioxygen results in the formation of prostaglandin G₂.

Cox-1 activity is crucially dependent on the proper orientation of fatty acids substrates within the cyclooxygenase active site made possible by ionic bonding with Arg¹²⁰. When arginine is replaced with another positively charged amino acid, lysine, Cox-1 is fully active. However, substitution with the neutral asparagine produces an enzyme with less than 50% activity and a 1000-fold increased K_m value (Mancini et al., 1995; Bhattacharyya et al., 1996), and substitution of Arg¹²⁰ with the negatively charged glutamate produces an inactive ovine Cox-1, or a human Cox-1 with only 5% activity.

Arg¹²⁰ is also necessary for the binding and inhibition by acidic cyclooxygenase inhibitors, which constitute the largest group of nonselective NSAIDs. Crystal structures obtained with Cox-1 complexed with flurbiprofen place the carboxylate of this acidic NSAID within hydrogen bonding distance of Arg¹²⁰ (Fig. 3C) (Picot et al., 1994). Mutational analysis confirms the importance of this interaction for inhibition (Mancini et al., 1995; Bhattacharyya et al., 1996). Substitution of Arg¹²⁰ in Cox-1 with glutamine or glutamate abrogates or reduces inhibition by the acidic NSAIDs indomethacin, flurbiprofen and ketoprofen, diclofenate, and meclofenamate (Fig. 1). Flurbiprofen, which inhibits native Cox-1 via a time-dependent mechanism, inhibits the R¹²⁰Q mutant only competitively, implying that interaction of the carboxylic acid moiety of the NSAID is also required for the conformation changes accompanying time-dependent inhibition by acidic (nonselective) NSAIDs (Bhattacharyya et al., 1996). Interestingly, nonacidic (Cox-2 selective) NSAIDs, such as Dup697 and L-746, 483, inhibit the R120E mutant about 10 times more potently than the native Cox-1, suggesting that the presence of Arg¹²⁰ may hinder binding of nonacidic inhibitors in Cox-1 (Mancini et al., 1995).

In contrast to its function in Cox-1, Arg¹²⁰ plays only an accessory role for NSAID and most fatty acid binding in Cox-2. The R¹²⁰Q Cox-2 mutant is fully active with arachidonate (W. L. Smith and C. J. Reike, personal communication), and the R¹²⁰E mutant has near full activity albeit with a 30-fold increased K_m value (Greig et al., 1997). Structures obtained from cocrystals of flurbiprofen and indomethacin suggest that some acidic inhibitors bind to Cox-2 in a manner similar to that of flurbiprofen in Cox-1 (i.e., aided by ionic interaction of their carboxylate moieties with Arg¹²⁰) (Kurumbail et al., 1996). Nevertheless, other acidic inhibitors (e.g., meclofenamic and diclofenac) only marginally depend on Arg¹²⁰, as demonstrated by the ability of these drugs to potentially inhibit the R¹²⁰E Cox-2 mutant (Greig et al., 1997). Furthermore, the positively charged arginine may actually discourage binding of some nonacidic inhibitors to Cox-2. The IC₅₀ values for NS398, DuP697, and SC58125 are decreased by as much as 1000-fold when neutral glutamine is substituted for arginine in the R¹²⁰Q Cox-2 mutant (W. L. Smith and C. J. Reike, personal communication).

The nonessential role of Arg¹²⁰ for NSAID and fatty acid binding in Cox-2 presumably results because this isozyme has a larger cyclooxygenase pocket than Cox-1 (Fig. 4). By comparing their relative crystal structures, the central channel of the NSAID-binding pocket of Cox-2 has been estimated to be about 17% larger than that of Cox-1 (Fig. 4) (Luong et al., 1996). This increased size may simply allow fatty acids and inhibitors to bind more tightly in the Cox-2 active site, reducing the relative importance of ionic interactions with Arg¹²⁰. Another consequence of the larger Cox-2 active site is that it may reduce charge and steric crowding by Arg¹²⁰ at the mouth of this pocket and thereby increase access, or permit more avid binding, of nonacidic inhibitors in Cox-2. Thus, Arg¹²⁰ may indirectly contribute to drug selectivity by discriminating against binding of nonacidic NSAIDs more effectively in the compact Cox-1-binding site than in the larger Cox-2.

Cox-2-Selective Inhibitors. The most critical structural features of Cox-2 that confer sensitivity to inhibition by selective NSAIDs are several amino acid changes that increase the size and chemical environment of the Cox-2 NSAID-binding pocket. Most important among these is the substitution of valine in Cox-2 for Iso⁵²³, an amino acid that lines the surface of the Cox-1 cyclooxygenase active site (Fig. 3D). This change to the smaller valine in Cox-2 permits access to a pocket, or nook, near the mouth and adjacent to the central channel of the binding pocket, increasing the volume of the Cox-2 NSAID-binding site many times beyond that resulting from the loss of a single methyl group (Fig 3D). A second valine substitution in Cox-2, this one for Iso⁴³⁴ within the second shell of amino acids that line the Cox-1 active site, increases the mobility of Phe⁵¹⁸, which allows this amino acid to swing out of the way, further increasing access to the side chamber. The larger main channel and extra nook combine to make the total NSAID-binding site about 25% larger in Cox-2 than in Cox-1 (Luong et al., 1996) (Fig. 4). This extra size is essential for selective inhibition of Cox-2 by NSAIDs because if access to this side chamber is restricted in Cox-2 by switching valine back to isoleucine, Cox-2 is no longer differentially sensitive to these inhibitors (Gierse et al., 1996; Guo et al., 1996).

A second essential amino acid switch between Cox-1 and Cox-2 changes the chemical environment within the binding pocket. That change involves the substitution in Cox-2 of arginine for histidine at position 513 within the side chamber made accessible by the Iso→Val switch. Interaction of the

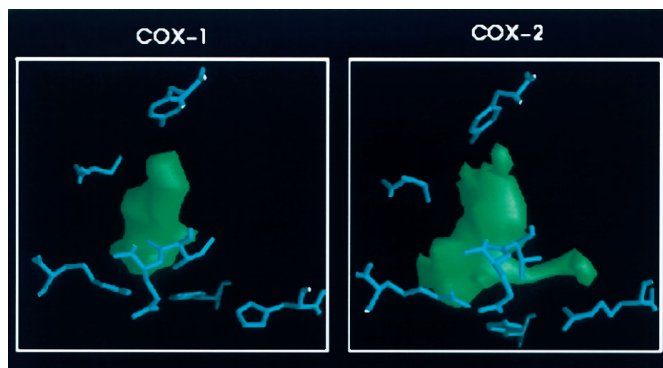


Fig. 4. Comparison of the accessible volume of the sheep Cox-1 and human Cox-2 hydrophobic substrate binding pockets (Luong et al., 1996).

4-methylsulfonylphenyl or 4-sulfonamoylphenyl substituents of diarylheterocyclic inhibitors with this arginine appears to be required for the time-dependent inhibition of Cox-2 by these inhibitors (Kurumbail et al., 1996). In vitro mutagenesis experiments with Cox-1 have shown that although the I⁵²³V Cox-1 mutant has a decreased IC₅₀ value for Cox-2-selective NSAIDs, the second H⁵¹³R mutation is required for full sensitivity to time-dependent inhibition by Cox-2-selective drugs (Wong et al., 1997).

Arg⁵¹³ may also promote binding and time-dependent inhibition by the zompirac-derived class of inhibitors. Instead of directly interacting with these inhibitors, it has been proposed that Arg⁵¹³ forms hydrogen bonds with two other amino acids residues in the NSAID-binding pocket, Glu⁵²⁴ and Tyr³⁵⁵, allowing the zompirac-based inhibitors to bind more tightly (Luong et al., 1996).

The important consequence of the amino acid changes in Cox-2 is to increase the size of the NSAID-binding pocket, allowing this isozyme to bind bulky inhibitors more readily than Cox-1. Structures of Cox-2 cocrystallized with SC588 indicate that the phenylsulfonamide moiety of this diarylheterocyclic compound fits into the extra nook made accessible by Val⁵²³ (Kurumbail et al., 1996) (Fig. 3D). Binding in this nook is probably also facilitated by Arg⁵¹³, which is within bonding distance of the sulfonamide group (Fig. 3D).

Ultimately, however, selectivity occurs because Cox-2 NSAIDs inhibit Cox-2 by a time-dependent slowly reversible mechanism, whereas they inhibit Cox-1 by a freely reversible competitive mechanism. Time-dependent inhibition occurs when the freely reversible cyclooxygenase-inhibitor complexes undergo a conformational change to form tightly binding complexes (Fig. 2). Depending on the concentration and NSAID used, it can take several seconds to minutes to reach equilibrium between the reversible and the pseudoirreversible enzyme-inhibitor complexes and to achieve full inhibition. Although these tightly binding complexes are not covalent, they dissociate only slowly and therefore effectively inactivate the enzyme. The practical result of this mixed mode of inhibition is that if the blood concentration of a selective NSAID is below the IC₅₀ value for Cox-1, the formation of enzyme-inhibitor complexes will only minimally inhibit Cox-1 activity but will lead to inactivation of most or all Cox-2 (Fig. 2).

The drug-protein interactions necessary for time-dependent inhibition are not well understood, and indeed they seem to vary among inhibitor classes and even between drugs within a given class. For most acidic time-dependent NSAIDs, interaction with Arg¹²⁰ appears to be required because these drugs inhibit the R¹²⁰Q and R¹²⁰E mutants only competitively (Mancini et al., 1995; Bhattacharyya et al., 1996). As mentioned, time-dependent inhibition by sulfonamide or methylsulfoxide containing Cox-2-selective NSAIDs appears to depend on interaction with Arg⁵²³ (Wong et al., 1997). Curiously, inhibition by the methylsulfoxide inhibitor NS-398 results from interaction with Arg¹²⁰ and not Arg⁵¹³. NS-398 binds in the Cox-2 active site, similarly to acidic NSAIDs (Marnett and Kalgutkar, 1998), and inhibits the R¹²⁰E Cox-2 mutant only competitively (Greig et al., 1997).

In summary, two amino acid changes within the cyclooxygenase active sites of Cox-1 and Cox-2 are primarily responsible and essential for the different sensitivities of these two isozymes for selective Cox-2 inhibitors (Fig. 3, C and D). The

first is the substitution of Iso→Val at position 523, which by providing access to a side chamber in Cox-2, increases the effective size of the Cox-2 active site relative to Cox-1 and permits this isozyme to bind bulkier NSAIDs than Cox-1. An additional Iso→Val substitution at position 434 in the second shell surrounding the Cox-2-binding pocket may contribute secondarily to selectivity by increasing the mobility of side chains within the pocket to further increase the effective size of the active site. The overall larger size of the central channel of the Cox-2 NSAID-binding pocket may also preferentially reduce steric and ionic crowding by the charged Arg¹²⁰ in Cox-2 and thus preferentially increase binding of non-acidic NSAIDs by this isozyme. The second essential amino acid change that results in Cox-2 drug sensitivity is the exchange of His⁵¹³ in Cox-1 for an arginine in Cox-2. This arginine is within bonding distance of the sulfonamide moiety in the crystal structures of Cox-2 with the diarylheterocyclic inhibitor SC588 (Kurumbail et al., 1996), and in vitro mutagenesis experiments confirm its importance for time-dependent inhibition by this class of inhibitors (Wong et al., 1997).

Outlook

The results of animal experiments with selective inhibitors and early clinical trials support the conclusion that Cox-2-selective drugs will be safer, just as effective, and thus an overall improvement over current nonselective inhibitors.

Nevertheless, there remains some health and safety concerns that should be monitored in patients using these new Cox-2 NSAIDs. One possibility is that the side effects that accompanied the old nonselective inhibitors, dyspepsia and gastric irritation, may have set the upper limit on dosage and long-term use of these drugs and that in the absence of these complications, new and unexpected side effects will become unmasked; in other words, gastrointestinal toxicity could turn out to be a useful warning indicator signaling the safe limits for cyclooxygenase inhibition.

One of the most troubling findings in the clinical trials has been that patients taking MK-966 (rofecoxib) (P. Emery, Second International Workshop on Cox-2, July 28–31, 1998) and SC58635 (celecoxib) had a slight increase in the incidence of edema, a condition often resulting from alterations in kidney function. NSAIDs are presently contraindicated in patients with renal insufficiency because their use can precipitate complete renal failure. It is not known whether the edema associated with these drugs is idiosyncratic or representative of all Cox-2 inhibitors. However, if the renal complications are found to result from generic Cox-2 inhibition, then the same care will have to be taken in prescribing Cox-2 inhibitors for the elderly and other patients with potential kidney ailments as has previously been taken with nonselective NSAIDs.

A more serious and widespread concern for Cox-2-selective inhibitors is that they may counteract the positive cardiovascular effects of aspirin and could even increase cardiovascular disease. Aspirin, when taken in small daily doses, reduces the incidence of fatal heart attacks and their recurrence by as much as 25% (Willard et al., 1992). These therapeutic benefits result because aspirin selectively blocks platelet Cox-1 and the resulting synthesis of thromboxane, a proaggregatory prostaglandin, without affecting endothelial cell production of prostacyclin, an antiaggregatory prostaglandin. Aspirin

is beneficial because it tips the balance of vascular prostaglandin production in favor of prostacyclin synthesis, thereby reducing thrombosis. Nonselective inhibitors block both Cox-1 and Cox-2 and thus do not affect the balance between prostacyclin and thromboxane synthesis; therefore, they have little cardiovascular effect. In contrast, selective Cox-2 inhibitors reduce prostacyclin synthesis by as much as 50% (F. Catella-Lawson, Second International Workshop on Cox-2, July 28–31, 1998) By design, these compounds do not affect platelet Cox-1 thromboxane synthesis and therefore may bias vascular prostaglandin synthesis in favor of thromboxane production, a prothrombotic outcome. Although studies examining the consequences of Cox-2 inhibition on vascular prostaglandin synthesis are still in their early stages, it seems wise in the future to monitor stroke and heart attack rates in patient populations taking these drugs.

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