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Cyclooxygenase-2 inhibitors: promise or peril?

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THE discovery of two isoforms of the cyclooxygenase enzyme, COX-1 and COX-2, and the development of COX-2-specific inhibitors as anti-inflammatories and analgesics have offered great promise that the therapeutic benefits of NSAIDs could be optimized through inhibition of COX-2, while minimizing their adverse side effect profile associated with inhibition of COX-1. While COX-2 specific inhibitors have proven to be efficacious in a variety of inflammatory conditions, exposure of large numbers of patients to these drugs in postmarketing studies have uncovered potential safety concerns that raise questions about the benefit/risk ratio of COX-2-specific NSAIDs compared to conventional NSAIDs. This article reviews the efficacy and safety profiles of COX-2-specific inhibitors, comparing them with conventional NSDAIDs.

Key words: nonsteriodal anti-inflammatory drugs (NSAIDs); COX-2-specific inhibitor; prostaglandins; rheumatoid arthritis; gastrointestinal toxicity; cardioprotection

Introduction

In 1971, Sir John Vane discovered that aspirin worked by inhibiting the action of cyclooxygenase (COX) (also termed prostaglandin endoperoxide synthetase) in synthesizing prostaglandins.[1] That discovery spurred the synthesis of several additional drugs that also inhibited cyclooxygenase, and had anti-inflammatory properties. To distinguish these drugs (including aspirin and other salicylates) from anti-inflammatory glucocorticoids, they were collectively termed nonsteroidal anti-inflammatory drugs (NSAIDs).

In the late 1980s and early 1990s, a series of discoveries were made that led to the identification of two COX enzymes: COX-1, which is a constitutively expressed isoform involved in physiologic maintenance functions; and COX-2, which is predominantly synthesized in response to inflammatory stimuli.[2–4] This recognition quickly resulted in the synthesis of compounds that specifically inhibit COX-2 while sparing COX-1, with the hopes that such compounds would be at least as efficacious as and safer than NSAIDs, which inhibited both COX-1 and COX-2.

NSAIDs can be placed into three categories with respect to inhibition of COX-1 and COX-2: conventional or non-selective NSAIDs, which either preferentially inhibit COX-1 or inhibit COX-1 and COX-2 at about the same plasma concentration; COX-2-selective NSAIDs, which preferentially inhibit COX-2, but at higher therapeutic concentrations can also inhibit COX-1; and COX-2-specific NSAIDs, which even at higher therapeutic concentrations inhibit only COX-2 and spare COX-1. While NSAIDs largely share the same therapeutic properties, the adverse effect profile of each NSAID depends in part on its behavior with respect to COX-1 and COX-2 inhibition.

Biochemistry and pharmacology of NSAIDs

Mechanism of action of NSAIDs

The COX enzyme has two distinct active sites, respectively termed the cyclooxygenase active site and the peroxidase active site. The cyclooxygenase site cyclizes arachidonic acid and adds a hydroperoxy group to carbon 15 to form prostaglandin G_2 (PGG_2). The separate peroxidase site of the same COX molecule then reduces this hydroperoxy group to the hydroxy group to form PGH_2. NSAIDs inhibit the cyclooxygenase active site of COX, but have no effect on the peroxidase active site, a finding confirmed by recent X-ray crystallographic evidence of COX incubated with selected NSAIDs.[5,6]

The identification of two COX isoenzymes, COX-1 and COX-2, prompted numerous investigations to define the structure–function relationship of each isoform. COX-1 and COX-2 are encoded by two different genes. The amino acid sequences of human COX-1 and human COX-2 are 63% identical and 78% similar. The cyclooxygenase active sites (and the NSAID binding sites) of human COX-1 and human COX-2 are highly conserved, differing by only a single

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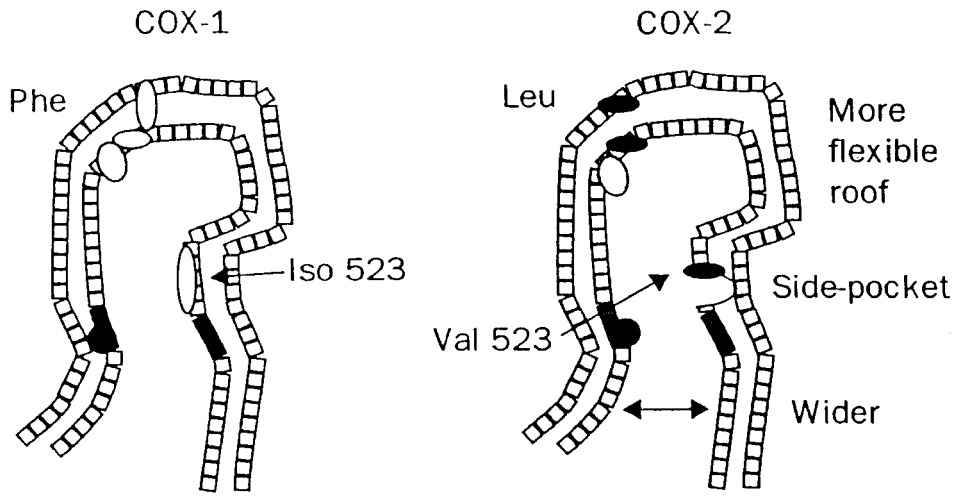

FIG. 1. Differences between the cyclooxygenase active sites of COX-1 and COX-2. The isoleucine at position 523 (Iso 523) in COX-1 is replaced by a valine in COX-2. This substitution creates a side pocket of the active binding site of COX-2, not present in COX-1, and a somewhat wider binding site in COX-2.

amino acid. Residue 523 is an isoleucine in COX-1 and a valine in COX-2 (Fig. 1). The absence of the additional methyl group on the valine in COX-2 compared with the isoleucine in COX-1 produces a side pocket as part of the NSAID binding site of COX-2, which is not present in the NSAID binding site of COX-1, and increases the overall size of the binding site.[7] Conventional non-selective NSAIDs fit the NSAID binding sites of both COX-1 and COX-2, and act as reversible competitive inhibitors of both enzymes. Aspirin acetylates the active cyclooxygenase site, forming a covalent bond, and thus acts as an irreversible inhibitor of both COX-1 and COX-2.

COX-2-specific inhibitors such as celecoxib and rofecoxib are larger than conventional NSAIDs. These specific inhibitors have a conformation that precludes them from readily fitting into the NSAID binding site of COX-1 but allows them to easily fit into the side pocket of the NSAID binding site of COX-2. This difference in part explains the COX-2 selectivity of these newer NSAIDs. In addition, however, kinetic studies have established two distinct mechanisms by which COX-2-specific NSAIDs inhibit COX-1 and COX-2. Specific COX-2 inhibitors such as celecoxib and rofecoxib show a time-dependent irreversible inhibition of COX-2, whereby the drug appears to alter the active cyclooxygenase site following binding. At very high doses, however, these same compounds act as time-independent reversible inhibitors of COX-1, with the degree of inhibition of COX-1 dependent on arachidonic acid concentration, drug concentration, and affinity for the active site.[8–10]

Tissue distribution of COX-1 and COX-2

COX-1 is constitutively expressed in most tissues, and appears to act in a homeostatic or cytoprotective manner. The tissues expressing COX-1 that demonstrate the most clinically relevant NSAID effects are the gastrointestinal (GI) mucosa, the kidneys, and platelets. (While the GI mucosa and the kidney can also express COX-2, platelets express only COX-1 and cannot be induced to express COX-2.) In contrast, COX-2 is absent from most tissues (it does appear to be constitutively expressed in brain, testes, and the kidney[11]), but can be induced in most tissues by cytokines, endotoxin, tumor promoters, growth factors, and gonadotropins.[2,3,12,13] After induction, COX-2 can be found in multiple cell types, including macrophages, monocytes, synoviocytes, ovarian follicles, colonic adenomas and cancer cells, vascular smooth muscle cells, bone, and amnion, and in increased amounts in the brain, spinal cord, and the kidney.

COX-1 and COX-2 have identical enzymatic actions, and synthesize PGH_2. Depending on the tissue where it is synthesized, PGH_2 can be converted to prostaglandins PGD_2, PGE_2, $PGF_{2\alpha}$, and PGI_2 (prostacyclin), and to thromboxane. In turn, the physiologic or pathophysiologic actions of each of these prostanoids are dependent on the microenvironment where it is made. PGE_2 synthesized by the cells of the gastric mucosa serves a cytoprotective role, while PGE_2 synthesized by the synovial lining of a rheumatoid arthritis joint is pro-inflammatory. Thromboxane A_2(TxA_2) synthesized by COX-1 in platelets promotes their aggregation. PGI_2 is synthesized at least in part by COX-2 within arterial walls, inhibits the aggregation of platelets, is a potent vasodilator, and opposes the actions of TxA_2. In addition, prostaglandins can act in an autocrine, paracrine, or endocrine manner. Inhibition by NSAIDs of COX-1-synthesized prostaglandins or thromboxanes appears to be responsible for many of the common adverse effects of NSAIDs,

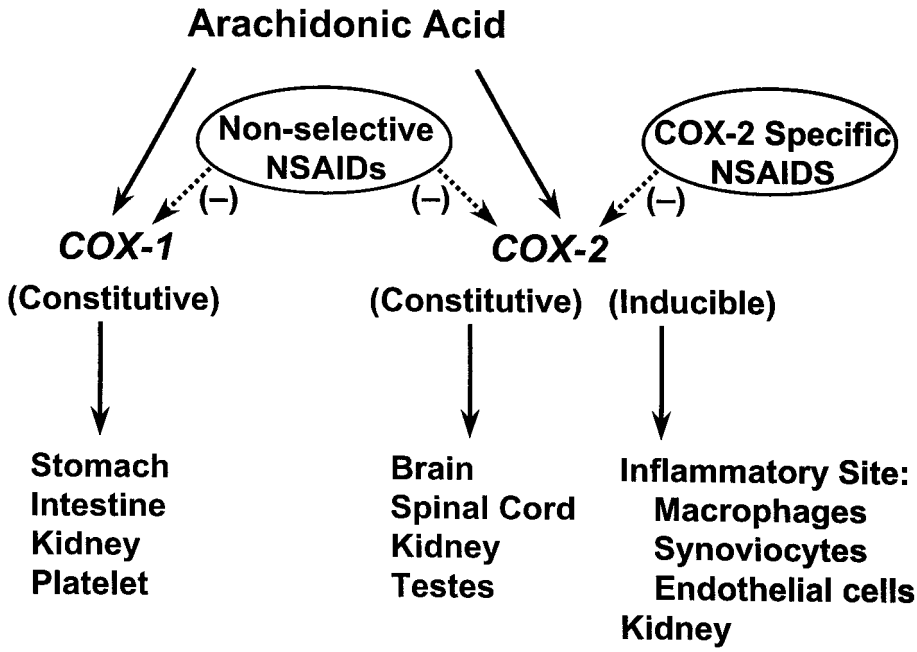

FIG. 2. The tissues where COX-1 and COX-2 are constitutively expressed and COX-2 is inducibly expressed, and where NSAIDs may exhibit clinical effects. The inhibitory actions of non-selective NSAIDs on COX-1 and COX-2, and the inhibitory actions of COX-2-specific NSAIDs on constitutive and inducible COX-2, are schematically illustrated.

such as stomach ulceration, renal effects, and inhibition of platelet aggregation. When prostaglandins are synthesized by induced COX-2 at particular tissue sites, their pro-inflammatory actions can result in erythema, edema, tenderness, pain and fever. Inhibition by COX-2-specific NSAIDs of COX-2-synthesized prostaglandins appears to be responsible for at least some adverse renal effects, and possibly some adverse cardiovascular effects[13] (Fig. 2).

Chemical properties of COX-2 specific NSAIDs

COX-2-specific NSAIDs are weak organic acids, and lipophilic. Thus, the lower the pH, the greater is their lipophilicity. This combination of chemical properties allows the COX-2-specific NSAIDs (as well as conventional NSAIDs) to cross lipid membranes, including the blood–brain barrier, and to accumulate in acidic tissues such as the stomach, renal medulla, and sites of inflammation.[14]

COX-2 selectivity

The COX-2 selectivity of NSAIDs is defined by the COX-1/COX-2 ratio. The larger this ratio, the greater the selectivity of the compound is for COX-2. However, this ratio can vary remarkably, depending on which of the different *in vitro* and *in vivo* assays is used to generate the ratio. Furthermore, it is important to realize that, especially for the *in vitro* assays, the assay conditions may vary from laboratory to laboratory, and thus the ratio for a given drug can vary over a wide numerical range depending on which laboratory is reporting the result.

The *in vitro* assay that determines the concentrations required to inhibit 50% of the activities (the IC_{50} values) of purified recombinant human COX-1 and purified recombinant human COX-2, respectively, is the most direct method for determining the specificity of a given compound for COX-2. However, because this assay does not take into account factors that occur *in vivo*, such as intracellular locations of COX-1 and COX-2, and potential differences in local intracellular drug concentrations, the COX-1/COX-2 ratio generated by this assay does not reflect the clinical situation. A second *in vitro* assay, the whole blood assay, incorporates some of the factors already noted, and examines the IC_{50} values needed to inhibit the synthesis of thromboxane (a purely COX-1-initiated event) synthesized by platelets during aggregation, and of PGE_2 elaborated following lipopolysaccharide stimulation of monocytes (a purely COX-2-mediated event).[15] The results of this assay, however, can be affected by the particular assay conditions used and, thus, again do not adequately reflect the clinical situation. The most relevant COX-1/COX-2 ratios are generated using *in vivo* assays. Such assays were developed in the rat, and were used to determine the dose required to inhibit 50% of the activities (the ED_{50} value) of COX-1 (as determined by synthesis of gastric prostaglandins) and of COX-2 (as determined

Table 1. COX-1/COX-2 ratios[a] for selected NSAIDS by various assays

Drug	Human recombinant enzymes[b] (COX-1 IC_{50}/COX-2 IC_{50})	Whole blood assay[c] (COX-1 IC_{50}/COX-2 IC_{50})	*In vivo*[d] (COX-1 ED_{50}/COX-2 ED_{50})
Non-selective (conventional) NSAID[5]			
Flurbiprofen	–[f]	0.097	–
Ketoprofen	–	0.123	–
Tolmetin	–	0.254	–
Aspirin	–	0.321	–
Oxaprozen	–	0.397	–
Naproxen	0.136	0.559	5
Indomethacin	0.1	0.562	2
Ibuprofen	0.215	0.592	0.1
Ketorolac	–	0.610	–
Piroxicam	1.026	1.266	0.1
Diclofenac	3	20	5
Mefenamic acid	–	12.5	–
Selective COX-2 inhibitors			
6-MNA	–	1.563	0.01
Etodolac	> 1.852	9.09	0.2
Meloxicam	0.765	11.1	0.5
Nimesulide	–	25	–
Specific COX-2 inhibitors			
Celecoxib	375	7.6–9.09	> 33
Rofecoxib	1000[g]	20–35	–
Valdecoxib	35,000	30[h]	–
Etoricoxib	–	106[8]	–

[a] Expressed as the ratio of the 50% inhibitory concentration or inhibitory dose for COX-1 to the 50% inhibitory concentration or inhibitory dose for COX-2. Ratios < 1 indicate preferential inhibition of COX-1, and ratios > 1 indicate preferential inhibition of COX-2. The larger the ratio, the more selective the drug. These ratios may vary by 10-fold depending on assay conditions.
[b] Data from preference 18.
[c] Data from preference 19.
[d] Data from preference 3.
[e] Designations of COX-2-non-selective, COX-2-selective, and COX-2-specific are based on the presence or absence of inhibition of COX-1-mediated events at therapeutic levels of NSAID that inhibit COX-2-mediated events *in vivo*.
[f] –, information not available.
[g] Data from reference 9.
[h] Data from reference 20.

by synthesis of prostaglandins induced in response to carrageenan injected into an air pouch), respectively.[16] The COX-1/COX-2 ratios for select NSAIDs and COX-2 inhibitors as determined by various assays are presented in Table 1.[17–20] Ultimately, however, the most meaningful classification of a NSAID with respect to COX-2 selectivity is that based on clinical criteria. On this basis, 'COX-2-specific' inhibitors inhibit only COX-2-mediated events, and not COX-1-mediated events even at high therapeutic doses. Clinical data suggest that celecoxib, rofecoxib, valdecoxib, and etoricoxib are 'COX-2-specific'. 'COX-2-selective' or 'COX-2-preferential' inhibitors inhibit COX-2-mediated events at low therapeutic doses, but inhibit COX-1-mediated events at higher clinically therapeutic doses. Clinical data suggest that meloxicam, nabumatone, and etodolac are 'COX-2-selective'. 'COX-2-non-selective' inhibitors at clinically relevant therapeutic doses inhibit COX-1-mediated events preferentially or inhibit COX-1- and COX-2-mediated events approximately equally. Most conventional NSAIDs fall into this category.

Clinical experience: efficacy of COX inhibitors

Roles of COX-1 and COX-2 in analgesic and anti-pyretic efficacy of NSAIDs

Local tissue injury and inflammatory diseases such as rheumatoid arthritis and osteoarthritis are associated with increased prostaglandin synthesis, which sensitizes pain receptors to lower levels of stimuli.[21] NSAIDs exert their analgesic effects by interfering with the hyperalgesia induced by local synthesis of prostaglandins at the site of injury or inflammation. In addition, prostaglandins are thought to act centrally to facilitate transmission of pain responses,[22], and NSAIDs can cross the blood–brain barrier to act at these central sites.[23] Studies in animal models have implicated both COX-1 and COX-2 in the release of centrally and peripherally acting prostaglandins, and have suggested that inhibition of both COX-1 and COX-2 may contribute to the spinal analgesic and anti-hyperalgesic actions of NSAIDs.[24,25] However, the relative roles of the two COX isoforms have not yet been completely defined. COX-2 expression is

increased locally at sites of injury and inflammation,[26] as well as in spinal cord neurons following peripheral inflammation.[27] At doses that maintain selectivity for COX-2, COX-2 inhibitors have shown equal analgesic efficacy to non-selective NSAIDs for treatment of pain associated with dental surgery, osteoarthritis, and dysmenorrhea.[26,28,29]

Exogenous pyrogens (e.g. bacterial endotoxin and viruses) induce fever via a cascade of molecular interactions that include induction of the synthesis and release of the endogenous pyrogenic cytokines interleukin (IL)-1 and IL-6, which in turn induce COX-mediated prostaglandin synthesis in the central nervous system.[30] Non-selective NSAIDs are effective anti-pyretics in both animal models and humans by inhibiting synthesis of fever-mediating prostaglandins. Recent biochemical and clinical data implicate the COX-2 isoform in the pathogenesis of fever in humans. COX-2 expression is induced in the brain vasculature, with temporal correlation to the development of fever,[31] and COX-2 knock-out mice fail to develop fever in response to inflammatory stimuli.[32] COX-2-specific inhibitors reduce naturally occurring fever in humans with efficacy similar to non-selective NSAIDs.[33]

Roles of COX-1 and COX-2 in anti-inflammatory efficacy of NSAIDs

NSAIDs are widely prescribed for and provide effective treatment of a variety of inflammatory conditions, including osteoarthritis, rheumatoid arthritis, acute gout, acute bursitis, and spondylarthropathies. Specific COX-2 inhibitors are indicated for osteoarthritis, rheumatoid arthritis, and management of acute pain.[34,35] Substantial individual variability exists with respect to the pharmacology and pharmacokinetics of NSAIDs and specific COX-2 inhibitors, necessitating individualization of treatment to the patient and disease. Furthermore, the optimum dosage varies from one disorder to the next. Moderate doses are often sufficient to treat osteoarthritis, whereas rheumatoid arthritis or other types of chronic inflammatory arthritis usually require sustained therapy with maximum tolerated doses.

Experimental evidence suggests that local PGE_2 production is central to the pathogenesis of inflammation.[26] Results obtained in animal models of inflammatory arthritis associate increased expression of COX-2 with increased prostaglandin production in inflamed joint tissues.[36] COX-2 is greatly upregulated at sites of inflammation in humans; induction of COX-2 expression has been observed in cartilage from osteoarthritis patients and synovial tissue from rheumatoid arthritis patients.[37,38] COX-2 expression in primary cultures of human synovial tissue or inflammatory cells (e.g. monocytes) is induced by the pro-inflammatory cytokines IL-1 and tumor necrosis factor-α, while the anti-inflammatory cytokines IL-4 and IL-13 and immunosuppressive glucocorticoids decrease COX-2 levels.[39] Clinical trials comparing COX-2-specific inhibitors with non-selective NSAIDs in osteoarthritis and rheumatoid arthritis have shown that the same therapeutic endpoints are reached with both types of drugs, suggesting that COX-2-specific inhibitors are equally efficacious as non-selective NSAIDs.[40,41]

Roles of COX-1 and COX-2 in efficacy of NSAIDs in cancer

Epidemiological data have shown an association between regular use of aspirin or other NSAIDs and decreased incidence of and mortality from colorectal cancer.[42–44] Regular aspirin use has been shown to decrease both colorectal cancer incidence and mortality by approximately 40%.[43,44] Sulindac administration has been shown to reduce the size and number of adenomas in patients with familial adenomatous polyposis (FAP), a hereditary disease that leads to colorectal cancer by the fifth decade of life in virtually all patients.[45] COX-2 has been implicated in carcinogenesis in animal models of the disease,[46,47] and COX-2 has been shown to be highly upregulated in human colorectal adenomas and adenocarcinomas compared with normal mucosa,[48] suggesting a role for COX-2 in transformation. However, no data are yet available demonstrating an association between NSAID administration and decrease in cancer incidence in patients with FAP. The COX-2-specific inhibitor celecoxib has been shown to significantly reduce the size and number of colorectal polyps in patients with FAP, and has been approved by the Food and Drug Administration as an adjunct to usual care for patients with FAP.[49] Increased prostaglandin synthesis via COX-2 upregulation in bladder cancer,[50] and other GI cancers,[51,52] suggests that COX-2-specific inhibitors may be useful in the prevention or treatment of these cancers as well.

Roles of COX-1 and COX-2 in effects of NSAIDs in Alzheimer's disease

Non-selective NSAIDs have been evaluated for the treatment and prevention of Alzheimer's disease with promising results. Studies have shown slower disease progression and cognitive decline in NSAID-treated patients compared with matched control populations.[53–55] Longitudinal studies have demonstrated that the relative risk of developing Alzheimer's disease is decreased in patients using NSAIDs and is associated with the duration of use.[55–57] Animal studies have shown that COX-2 is localized during development to areas of the brain related to memory (e.g. hippocampus, cortex),[58], and that COX-2 appears to be involved in postsynaptic signaling of cortical and other excitatory neurons in the adult brain.[59] COX-2 expression in the brain can be upregulated by a variety of stressful stimuli, including seizure.[58] Other

animal studies have shown upregulation of COX-2 in activated microglia in cerebral inflammatory processes thought to parallel the inflammatory cascade of events in the human brain that lead to deposition of β-amyloid protein, the hallmark histologic manifestation of Alzheimer's disease.[60] These clinical and biochemical data suggest a potential for COX-2-specific NSAIDs in the future treatment and prevention of Alzheimer's disease.

Clinical experience: toxicities associated with COX inhibition

Although inhibition of both COX-1 and COX-2 is generally well tolerated, it is associated with a wide spectrum of potential clinical toxicities. In general, adverse events tend to be dose related.[61] Many adverse events are attributed to inhibition of the constitutively expressed COX-1 enzyme, and some of these appear to be significantly reduced through the use of COX-2-specific inhibitors. Other of these adverse events, however, are not reduced by the use of COX-2-specific inhibitors. Moreover, whether COX-2-specific inhibitors cause their own set of adverse events is emerging as an area of controversy.

COX-1 and COX-2 inhibition in GI toxicity

The chief clinical limitation of non-selective NSAIDs is undoubtedly their toxic effect on the upper GI tract, including ulceration, bleeding, obstruction, and perforation. Non-selective NSAIDs are thought to exert both a direct toxic effect on the gastroduodenal mucosa as well as an indirect effect via inhibition of COX-1-induced cytoprotective prostaglandins. Prostanoids PGE_2 and PGI_2, synthesized in the GI mucosa, protect the mucosa and limit gastric acid output. Central mucosal defense mechanisms are compromised by the decreased prostaglandin production caused by chronic NSAID-induced COX-1 inhibition, leading to ulceration and bleeding diathesis. NSAID-induced inhibition of COX-1 leads to increased gastric acid production, decreased production of bicarbonate, and a decreased rate of cellular proliferation of the gastric mucosa, all of which impair the normal protective mechanisms of the stomach. NSAIDs cause or aggravate GI bleeding, both by increasing acid production in the stomach and by decreasing platelet adhesiveness (see later).[62,63]

Post-marketing surveillance and endoscopic studies have confirmed that the incidence of gastroduodenal mucosal injury is reduced with the use of nabumatone, etodolac, and meloxicam, in part due to their selectivity for COX-2 inhibition, with a minimal effect on COX-1, at least at lower therapeutic doses.[64,65] Specific COX-2 inhibitors have also been shown by endoscopy to markedly reduce injury to the gastroduodenal mucosa. Two of these compounds, celecoxib and rofecoxib, have been extensively studied by endoscopy and appear to maintain their selectivity for COX-2 at doses substantially higher than those required to affect inflammation. In these studies, the incidence of endoscopic gastroduodenal ulcers attributable to specific COX-2 inhibitors is not significantly different from that found with placebo,[66,67] and is dramatically less than that seen with conventional NSAIDs.

However, whether a difference exists between non-selective NSAID inhibition and COX-2-specific inhibition in protection from clinically significant GI events is not at all as clear. The US Food and Drug Administration estimates that clinically significant GI events resulting from ulceration, including perforation, obstruction, and bleeding, occur in approximately 1–2% of patients using non-selective NSAIDs for 3 months and in approximately 2–5% of patients using them for 1 year. Two to four per cent of patients on long-term therapy are hospitalized each year because of GI complications.[68] Prior peptic ulcer disease, advanced age, high NSAID doses or therapy with multiple NSAIDs, and concomitant therapy with either corticosteroids or anticoagulants all increase the risk of a GI complication in patients taking non-selective NSAIDs chronically. There are some data indicating that comorbidities such as cardiovascular disease and rheumatoid arthritis increase the risk of NSAID-induced gastrointestibnal complications. It was hoped that the use of COX-2-specific inhibitors would decrease this incidence.

Two large trials have been conducted. The first, the CLASS trial,[69,70] compared celebrex with diclofenac and ibuprofen in osteoarthritis and rheumatoid arthritis patients. Patients were allowed to take up to 325 mg of aspirin per day for cardiovascular protection. The 12-month results of the trial showed no statistically benefit of celecoxib over diclofenac in protection from clinically significant GI events in patients overall, or in either the subset of patients who took aspirin or who did not take aspirin. Furthermore, there was no statistical benefit of celecoxib over ibuprofen in protection from clinically significant GI events in patients overall. Celecoxib did appear to have a statistically significant benefit over ibuprofen in patients not on aspirin, but, paradoxically, ibuprofen had a statistically significant benefit over celecoxib in patients who were taking aspirin. A possible explanation for this paradoxical finding has recently emerged[71] (see later). In the second trial, the VIGOR trial,[72] rofecoxib was compared with naproxen in rheumatoid arthritis patients only. No aspirin use was allowed. Rofecoxib demonstrated a statistically significant benefit over naproxen with respect to significant GI events, but there was an unexpected statistically significant increase in cardiovascular events in patients on rofecoxib compared with patients on naproxen (see later).

COX-1 and COX-2 inhibition in platelet and cardiovascular effects

Platelets have only the COX-1 isoform of cyclooxygenase, and use COX-1-derived PGH_2 to generate TxA_2, a key autocrine stimulator of platelet aggregation and vasoconstriction. Non-selective NSAIDs, by inhibiting COX-1 and thus platelet TxA_2 synthesis, inhibit platelet function and can exacerbate bleeding in patients who are otherwise at risk.[73] Because platelets lack mitochondria and are unable to synthesize additional cyclooxygenase, acetylation of this enzyme by aspirin irreversibly inhibits platelet activation in response to a variety of stimuli. This effect persists for 10–12 days until the acetylated platelets are replaced by newly produced platelets that have not been exposed to aspirin.[74] This property has led to the use of aspirin in doses as low as 80 mg daily in cardiovascular prophylaxis to prevent platelet aggregation and emboli in patients with a history of a myocardial infarction, angina, cerebrovascular accident, transient ischemic attack, angioplasty, and coronary bypass, but it also increases the risk of GI and other bleeding events. In contrast to aspirin, cyclooxygenase inhibition by other non-selective NSAIDs is reversible, and their platelet effects correlate roughly with the half-life of the drug, lasting only as long as the drug is present. COX-2-specific NSAIDs completely spare platelet function at therapeutic doses,[75] and do not interfere with the effect of aspirin on platelets when co-administered with aspirin.[71] However, recent data suggest that ibuprofen[71] (and possibly other conventional NSAIDs), when administered concurrently with aspirin, blocks aspirin from reaching the COX-1 binding site, thereby diminishing the effect of aspirin on inhibition of TxA_2 production, and abrogating the effect of aspirin on inhibition of platelet aggregation. Extrapolation of this observation to COX-1 in the GI mucosa may explain the paradoxical effect of ibuprofen on lowering the ulceration rate in the CLASS study.[69,70]

Aspirin and other non-selective NSAIDs do not cause major bleeding events in the vast majority of patients who use them. The clinical manifestations induced by exposure to non-selective NSAIDs are mild, in part because TxA_2 is only one of several mediators of platelet activation. Thrombin and other strong platelet agonists can induce platelet aggregation even in the presence of concomitant exposure to non-selective NSAIDs.[76] However, in patients with impaired hemostasis, the decreases in platelet TxA_2 resulting from COX-1 inhibition can pose significant clinical risk.

GI bleeding is the most common spontaneous bleeding event associated with the use of aspirin and non-selective NSAIDs. Other clinical bleeding problems associated with the use of non-selective NSAIDs are increased risk of intracerebral hemorrhage, fetal and neonatal bleeding abnormalities caused by maternal ingestion of NSAIDs in the peripartum period, and increased risk of post-operative bleeding following cardiac and other surgeries in patients taking aspirin and other NSAIDs preoperatively.[77] COX-2-specific NSAIDs, which have been shown to preserve platelet function at therapeutic doses, obviate these bleeding problems, and their use should not need to be restricted perioperatively.[75]

However, the preservation of platelet function by COX-2-specific NSAIDs may pose an increased risk for cardiovascular events in patients who use these drugs, and are already at risk for such events. As already noted, the VIGOR trial[72] compared rofecoxib with naproxen in rheumatoid arthritis patients, who were not permitted to take aspirin. While cardiovascular events were not a primary endpoint of the trial, they were monitored and reported. There was a statistically significant increase in the number of myocardial infarctions in the patients in the rofecoxib arm of the trial compared with patients in the naproxen arm of the trial. While patients at risk for cardiovascular events should have been excluded from the trial, a retrospective analysis indicated that a small subset of patients in the trial met the criteria of the Food and Drug Administration for use of aspirin for secondary cardiovascular prophylaxis, but were not taking aspirin. These patients, representing 4% of the study population, accounted for 38% of myocardial infarctions that occurred during the trial. There are three possible explanations for the finding. First, it may be a statistical aberration because the numbers are relatively small; second, naproxen, because of its non-selectivity and inhibition of COX-1, may be protective against such cardiovascular events; and third, rofecoxib may be predisposing to such events. In this third scenario, it has been hypothesized that rofecoxib is inhibiting COX-2-induced synthesis of prostacyclin by the vasculature, preventing vasodilatation and antagonism of platelet aggregation, thus leaving the effects of COX-1-induced platelet aggregation unopposed (see earlier). It should also be noted that the VIGOR trial studied solely patients with rheumatoid arthritis, which by itself may be a risk factor for cardiovascular events.[78,79] No such increase in cardiovascular events was seen in the CLASS trial, which studied both osteoarthritis and rheumatoid arthritis patients, and allowed aspirin use. Indeed, a retrospective analysis of the CLASS trial data comparing all patients taking celecoxib with those taking ibuprofen and diclofenac, and comparing non-aspirin users taking celecoxib with those taking ibuprofen and diclofenac, showed no statistically significant increase in the incidences of serious cardiovascular thromboembolic events between the celecoxib and NSAID comparators (combined or individually) for all patients, as well as the subgroup of patients not taking aspirin.[80] The explanation for the apparent discrepancy between the incidences of

cardiovascular events seen with rofecoxib and celecoxib appears not due to the different comparators used in the CLASS[69,70] and VIGOR[72] trials, and at this time remains unclear.

COX-1 and COX-2 inhibition in renal toxicity

NSAIDs can adversely affect renal function by inhibiting synthesis of renal prostaglandins important for solute homeostasis and for maintenance of renal blood flow. The most significant clinical effects of NSAIDs are decreased sodium and potassium excretion and decreased renal perfusion. NSAIDs, by decreasing renal PGE_2 levels, increase sodium reabsorption, causing weight gain and edema in some patients and, in rare cases, congestive heart failure. In addition, the effect of NSAIDs on sodium retention can decrease the response to anti-hypertensive drugs, particularly diuretics and angiotensin-converting enzyme inhibitors. Inhibition of renal PGI_2 by NSAIDs decreases potassium excretion and can cause hyperkalemia, usually mild but in rare cases sufficiently severe to cause cardiac arrest. Patients at risk for hyperkalemia (e.g. those with renal insufficiency or on potassium-sparing diuretics) should have their serum potassium levels monitored at the onset of NSAID therapy, since decreases in potassium secretion can occur with the first dose of NSAID. While maintenance of renal blood flow is independent of renal prostaglandin synthesis in healthy individuals, in clinical conditions where actual or circulating volume is decreased (congestive heart failure, cirrhosis, and renal insufficiency), renal perfusion is maintained by renal prostaglandins responsible for vasodilatation. NSAID administration to at-risk patients can result in decreases in renal blood flow sufficient to cause acute renal failure. Since acute renal failure can develop with the first dose of NSAID, careful monitoring of at-risk patients is important. It is likely that the risk of developing acute renal failure from NSAID therapy is both dose and half-life related, with higher doses of longer-acting NSAIDs associated with greater risk.[81] Whether there are differences among NSAIDs for risk of acute renal failure is unclear. However, a recent study suggested that use of high dose aspirin is more common in patients with chronic renal failure, then is use of other NSAIDs[82] Specific COX-2 inhibitors were not examined in the present study. Nonetheless, both non-selective and COX-2-specific NSAIDs should be used with appropriate monitoring in patients at risk for NSAID-induced adverse renal events. Because both COX-1 and COX-2 are expressed in the kidney,[83] and the relative physiological roles of each enzyme in the kidney have not been fully elucidated, the extent to which these adverse clinical effects might be obviated by COX-2-specific inhibitors has not been fully determined. COX-2-specific inhibitors have been shown to inhibit some renal prostaglandins,[84] and cause edema in 2–4% of patients in controlled clinical trials,[34,35] indicating that both non-selective and COX-2-specific inhibitors may have similar effects on sodium homeostasis.[85] However, there has been at least one report suggesting that celecoxib results in a lower incidence of hypertension and edema than rofecoxib.[86] This decreased incidence may be related to the shorter half-life of celecoxib than of rofecoxib (11 h versus 17 h), potentially allowing periodic synthesis of renal prostaglandins that help maintain renal function. It is also possible that the decreased incidence of hypertension observed with celecoxib in this study may be attributable to the blood pressure being monitored only once a day just prior to dosing, when drug plasma levels are at their nadir at steady state, rather than at several times during the dosing period. Further studies will be necessary to discern whether differences exist between COX-2-specific inhibitors with respect to renal effects.

COX-1 and COX-2 inhibition in hepatic toxicity

Hepatic toxicity has been reported with virtually all NSAIDs in current use. Most of this toxicity is clinically mild and seems to be unrelated to the inhibition of COX. Reversible hepatocellular toxicity, characterized by elevation of aminotransaminases, has been observed in up to 15% of patients treated with NSAIDs.[87] High anti-inflammatory doses of aspirin may cause this effect in up to 50% of patients, and even more frequently in patients with Still's disease and systemic lupus erythematosis (SLE). Transaminase elevations can occur at any time after initiation of treatment, and usually revert to normal with dosage adjustment or discontinuation of the drug. In rare cases, NSAIDs may induce more severe hepatic dysfunction, causing elevated bilirubin or prolonged prothrombin times, in which case the drug must be discontinued.

Clinically significant events reflecting NSAID hepatotoxicity are uncommon. It has been estimated from case–control studies that NSAID therapy increases the risk of hospitalization for acute symptomatic hepatitis by approximately two-fold.[88] Hepatocellular cholestasis and granulomatous hepatitis induced by phenylbutazone may be fatal in some patients. The most commonly reported hepatotoxicity of clinical significance occurs with sulindac. This drug is associated with a syndrome that appears to be allergic in nature, characterized by fever, rash, eosinophilia, and liver enzyme elevations. The hepatic toxicities observed with NSAIDs do not seem to result from inhibition of COX; therefore, they would not be expected to be more or less likely with the use of COX-2-specific inhibitors. COX-2-specific inhibitors have not been fully evaluated in patients with severe hepatic disease and should therefore be used with the same caution as the non-selective NSAIDs.

COX-1 and COX-2 in central nervous system toxicity

Both COX isoforms are present in the central nervous system, in neurons as well as vascular and glial cells. NSAIDs have been associated with central nervous system side effects. Salicylates can cause dose-dependent tinnitus and hearing loss, and overdoses can severely affect the central nervous system, culminating in coma and death; overdoses of other NSAIDs are much less toxic. Occasionally, some patients experience severe headaches on initiation of NSAID therapy; this effect may be more common with indomethacin than with other NSAIDs, and has been seen with both specific COX-2 inhibitors, celecoxib and rofecoxib.[34,35] Confusion may appear in elderly patients treated with indomethacin, naproxen, or ibuprofen. Aseptic meningitis has occurred rarely in patients treated with NSAIDs, most commonly ibuprofen.[61] It has been reported primarily in patients who have an underlying autoimmune disease, such as SLE or mixed connective tissue disease, and recurs with rechallenge using the same medication, but not with alternative NSAIDs, indicating that this is an idiopathic response to an individual drug not a mechanism-based effect.

COX-1 and COX-2 inhibition in cartilage and bone

Studies using non-selective NSAIDs have shown that these drugs may accelerate the process of cartilage destruction in osteoarthritis, largely by inhibiting proteoglycan biosynthesis in cartilage.[89,90] Potent inhibitors of prostaglandin synthesis, such as indomethacin and aspirin, have been shown to reduce proteoglycan synthesis in cartilage and promote the acceleration of joint space narrowing observed radiologically in patients with osteoarthritis.[89,90] Interestingly, indomethacin is one of several NSAIDs shown to lack collagenase inhibitory activity, and it may be that the combined inhibition of proteoglycan biosynthesis and lack of inhibition of collagenase activity allow for acceleration of cartilage destruction by indomethacin. Of note, the COX-2-selective inhibitor, meloxicam, shows no inhibitory effects on proteoglycan biosynthesis, but does inhibit collagenase activity, so would be predicted to be cartilage sparing; however, the effects of meloxicam on the progression of joint changes in osteoarthritis have not yet been definitively tested.[91]

The effects of NSAID therapy on bone proliferation and remodeling are complex and often contradictory. Prostaglandins can both stimulate collagen synthesis and bone formation, as well as promote bone resorption.[92,93] It has been reported that NSAIDs suppress bone repair and remodeling,[94] and also slow the process of bone loss associated with some inflammatory conditions.[95] Studies on the effects of non-selective NSAIDs in animal models of osteoporosis have given conflicting results, showing both protection against and acceleration of bone loss.[96,97] COX-1 is expressed in osteoarthritis cartilage and appears to be important in the repair of both cartilage and bone.[98] The effects of COX-2 inhibitors on cartilage and bone structure have recently been examined. In *in vitro* systems, celecoxib added to tissue cultures of osteoarthritic cartilage normalized proteoglycan turnover,[99] and rofecoxib added to tissue cultures of osteoarthritic cartilage inhibited IL-1-induced matrix metalloproteinase-1, matrix metalloproteinase-3, and nitric oxide production, and reversed IL-1 inhibition of cartilage synthesis,[100] suggesting that specific COX-2 inhibition may be chondroprotective. However, in an *in vivo* drug test chamber model in rabbits, rofecoxib administered orally suppressed bone formation,[101] raising the possibility that specific COX-2 inhibition could delay fracture healing. Thus, it appears that the net observed effect of a particular NSAID on bone and cartilage structure results from a complicated interplay of COX-1- and COX-2-controlled prostaglandin-mediated resorptive and formative processes.

Drug interactions of conventional and COX-2-specific NSAIDs

While individual NSAIDs vary in their interactions with other classes of drugs, many of these interactions have their basis in the mechanism of action of NSAIDs, and are common to many, if not all, NSAIDs.[85] Aspirin, in other than cardiovascular protective doses, should not be used together with other NSAIDs, as the combinations increase the potential for adverse effects common to this class. In addition, ibuprofen (and possibly other conventional NSAIDs) may diminish or abrogate the cardiovascular protective effect of aspirin when the two drugs are administered concurrently.[71] In contrast, COX-2-specific inhibitors do not abrogate the cardiovascular protective effects of aspirin.[71] Aspirin and NSAIDs may also share the same protein binding sites, and either aspirin can displace the NSAID, resulting in increased clearance and decreased plasma levels of the NSAID, or the NSAID can displace aspirin. Some NSAIDs, including aspirin, can cause displacement of other drugs from their protein binding sites, so caution needs to be used with patients on sulfonylurea hypoglycemics, phenytoin, valproic acid, and carbonic anhydrase inhibitors. Some, but not all, NSAIDs can also displace warfarin. Even with NSAIDs that do not displace warfarin, patients on warfarin and a NSAID need to have their protimes and/or International Normalized Ratios monitored. The interference of NSAIDs with synthesis of renal prostaglandins can blunt the natriuretic effects of loop and

thiazide diuretics, and can diminish the anti-hypertensive effects of angiotensin-converting enzyme inhibitors and β-blockers. Many, but not all, NSAIDs can cause decreased renal clearance and increased plasma levels of lithium, digoxin, cyclosporin A, and methotrexate. Patients on these drugs together with an NSAID should be monitored closely for toxicity. Antacids may interfere with absorption and therefore decrease plasma concentrations of some NSAIDs. Probenecid may decrease renal clearance and increase blood levels of some NSAIDs.

The COX-2-specific NSAIDs share most of the same concerns regarding drug interactions. In addition, it is known that celecoxib is metabolized by cytochrome P450 2C9, so any inhibitor of this enzyme should be used with caution in patients on celecoxib. In particular, administration of fluconazole at 200 mg per day resulted in a two-fold increase in the plasma concentration of celecoxib. In contrast to most NSAIDs, co-administration of celecoxib and methotrexate does not appear to increase methotrexate levels. Rofecoxib is metabolized primarily by cytosolic enzymes, and not by the P450 system. However, co-administration of rifampin with rofecoxib results in a 50% decrease in rofecoxib plasma concentrations, primarily through induction by rifampin of general hepatic metabolic activity.

Conclusions

NSAIDs are the most widely prescribed class of drugs. They are effective as analgesics and anti-inflammatories for a wide variety of clinical indications, but are accompanied by a wide spectrum of mechanism-based side effects. The identification of two isoforms of cyclooxygenase, COX-1 and COX-2, has led to the demonstration that COX-1 is constitutively expressed in most tissues and is homeostatic, and that COX-2 can be induced by a number of stimuli, and is largely responsible for inflammation, increased pain, and fever. Recent advances by pharmaceutical companies have led to the development of COX-2-specific inhibitors, which now command over $6 billion in annual revenue. The initial hope for these drugs was the same efficacy as conventional NSAIDs, but with a better safety profile. However, recent results from clinical trials have raised questions as to whether these drugs are truly safer. One large trial of a COX-2-specific NSAID has not demonstrated any advantage over conventional NSAIDs with respect to protection from clinically significant GI events, while a second large trial of a COX-2-specific NSAID has demonstrated an increased risk of myocardial infarction compared with a conventional NSAID. In contrast, a third study has suggested that COX-2-specific inhibitors do not interfere with the cardioprotective effects of aspirin. Additional COX-2-specific NSAIDs are currently in development. Whether this subset of NSAIDs proves to be any safer than conventional NSAIDs awaits the results of further clinical trials.

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