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CYCLOOXYGENASE-2: A Therapeutic Target

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■ **Abstract** Cyclooxygenase (COX), also known as prostaglandin endoperoxide synthase, is the key enzyme required for the conversion of arachidonic acid to prostaglandins. Two COX isoforms have been identified, COX-1 and COX-2. In many situations, the COX-1 enzyme is produced constitutively (e.g., in gastric mucosa), whereas COX-2 is highly inducible (e.g., at sites of inflammation and cancer). Traditional nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit both enzymes, and a new class of COX-2 selective inhibitors (COXIBs) preferentially inhibit the COX-2 enzyme. This review summarizes our current understanding of the role of COX-1 and COX-2 in normal physiology and disease.

INTRODUCTION

Prostaglandin endoperoxide synthase, commonly called cyclooxygenase (COX), is the key enzyme required for the conversion of arachidonic acid to prostaglandins. The two known COX isoforms are referred to as COX-1 and COX-2 for the order in which they were discovered. Aspirin, which works by inhibiting COX activity, has been available to the public for over 100 years; in fact, extracts from willow bark and myrtle, containing salicylates or their precursors, were prescribed by physicians for pain and fever centuries ago. However, only since 1971 has our understanding of the role of the COX enzyme in biology and disease become more clear.

Despite the wide use of nonsteroidal anti-inflammatory drugs (NSAIDs) over the past century, their mechanism of action was not fully appreciated until Vane (1) published his seminal observations indicating that the ability of NSAIDs to suppress inflammation is probably due to their ability to inhibit the COX enzyme. This effectively limits the production of proinflammatory prostaglandins (PGs) at a site of injury. Following this discovery, scientists and clinicians have used NSAIDs to dissect the critical role of both the COX enzymes and the eicosanoids derived from this pathway in normal physiology and disease states. It is important to note

that inhibition of the COX enzyme occurs at a drug concentration in the nanomolar to micromolar range. When NSAIDs and COX-2 selective inhibitors (COXIBs) are given at much higher doses, achieving concentrations of >100 μM, their effects are probably due to modulation of COX-independent signaling pathways.

Given the broad role of PGs in normal human physiology, it is not surprising that systemic suppression of PG synthesis through inhibition of COX can lead to unwanted side effects (Table 1). It is well-known that individuals taking NSAIDs for even short periods of time can experience severe gastrointestinal and renal side effects (2, 3), in addition to effects on other physiological systems. As many as 25% of individuals using NSAIDs experience some side effect and up to 5% develop serious health consequences.

The different effects of PGs can be explained by their varied chemistry, the diversity of PG receptors, and modulation of PG synthesis. The structural, cellular, and molecular biology of COX (4) and of prostanoid receptors (5) have recently been reviewed. Here, we focus on the role of COX-1 and COX-2 on the biology of different organ systems. Intensive research in the past 10 years has evaluated the relative contribution of each isoform. Because NSAIDs have proven efficacy in treating arthritis and pain yet can also cause deleterious side effects, a major goal of the pharmaceutical industry was to design an anti-inflammatory drug with a wider therapeutic window that lacked the serious side effects of non-selective NSAIDs. This led to the development of COXIBs, of which celecoxib (Celebrex) and rofecoxib (Vioxx) have dominated the U.S. market.

TABLE 1 Known and potential processes involved with COX-2 upregulation

Inflammation	Urogenital disease
Pain	Alzheimer's disease
Fever	Cancers:
Ovulation, pregnancy, and childbirth	Familial adenomatous polyposis
Renal function	Colorectal
Bone metabolism	Prostate
Tissue repair	Pancreatic
Myocardial infarction	Skin
Stroke	Head and neck
Atheroma	Esophagus
Diabetes	Breast
Diabetic retinopathy	Lung
Allograft rejection	

DISCOVERY OF AN INDUCIBLE CYCLOOXYGENASE

The COX-1 cDNA was initially isolated in 1988 from sheep, mouse, and human sources. The gene is 25 kb in size, is located on human chromosome 9q32–q33.3 (6), contains 11 exons (7), and produces a 2.8-kb mRNA (8) and a ∼68-kDa protein. Investigators evaluating a variety of signaling pathways identified a unique, inducible gene product that was homologous to the known COX-1 sequence (reviewed in 9). Others evaluating PG production in response to cytokines and other inflammatory mediators noted increases in COX activity probably due to increased expression of another COX (10). Immunoprecipitation of this COX variant with an anti-COX antibody, as well as the production of an antibody that precipitated only the COX-2 isoform, indicated the possibility of two different COX isoforms. Later, it was determined that the COX-1 and COX-2 proteins are derived from distinct genes that diverged well before birds and mammals (11). COX-2 is an 8-kb gene composed of 10 exons located on human chromosome 1q25.2–q25.3 (6). The mRNA is 4.1–4.5 kb (9) and encodes a protein of ∼68 kDa.

Both enzymes carry out essentially the same catalytic reaction and have similar tertiary structures (12), but the proinflammatory role appears to be mediated mainly by COX-2, whereas most of the "housekeeping" functions appear to be regulated by COX-1 (Figure 1). For the most part, each isoform's apparent function is consistent with its tissue expression pattern. Nearly all normal tissues express COX-1 with low to undetectable levels of COX-2, whereas COX-2 is constitutively expressed in the brain, pancreatic islet cells, ovary, uterus, and kidney (13). Other differences between COX-1 and COX-2 include differences in utilization of arachidonic acid substrate pools as well as in mRNA stability (14–16).

Joints

INFLAMMATION AND ARTHRITIS Although the role of COX activity in the production of PGs has been known since 1967 (17), the inducibility of this activity and the central role of this induction in inflammation have been elucidated only recently (18). Studies from animal models of inflammatory arthritis strongly suggest that increased expression of COX-2 is responsible for the increased PG production seen in inflamed joint tissues (19). COX-2 induction has been observed in both human osteoarthritis-affected cartilage (20) and synovial tissue from rheumatoid arthritis patients (21). Cell culture experiments utilizing primary cells derived from human synovial tissue or other cells, such as monocytes, have advanced our understanding of the regulation of COX-2 expression. The proinflammatory agents IL-1,TNF-α, and lipopolysaccharide, as well as the growth factors TGF-β, EGF, PDGF, and FGF,[1] have all been shown to induce COX-2 expression in this

[1]IL, interleukin; TNF, tumor necrosis factor; TGF, transforming growth factor; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor.

system. On the other hand, the anti-inflammatory cytokines IL-4 and IL-13, as well as the immunosuppressive glucocorticoids, were shown to decrease COX-2 levels (22).

Although the synovial tissues of patients with osteoarthritis express lower amounts of COX-2, primary explant cultures of human osteoarthritis-affected cartilage have been found to contain significant levels of COX-2, which produces

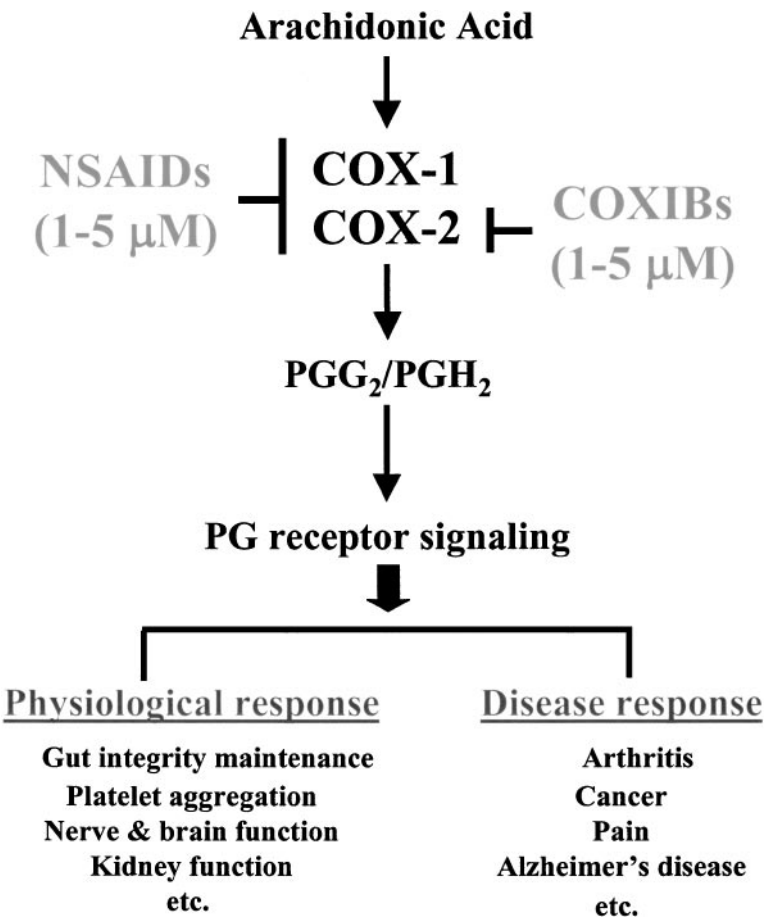

Figure 1 Schematic diagram of prostanoid signaling. COX-1 or COX-2 mediates the synthesis of PGG_2 and PGH_2 from arachidonic acid in a two-step reaction. PGH_2 is then metabolized by specific PG synthases to the 2-series prostanoids. Signaling through specific prostaglandin receptors mediates cellular responses in both physiological and disease states. Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit both COX-1 and COX-2, whereas specific COX-2 inhibitors (COXIBs) inhibit COX-2 with improved safety in the gut.

measurable quantities of PGs (20). Nitric oxide, another important inflammatory modulator, has been shown to regulate PG production in osteoarthritic cartilage, though not in synovial cells. Whether this modulation attenuates or enhances COX activity remains uncertain, although cross-talk between inducible nitric oxide synthase (iNOS) and COX-2 has been reported (23) and may be important in the development of osteoarthritis. Recently, investigators have shown that decreased production of nitric oxide through the selective inhibition of iNOS by N-iminoethyl-L-lysine significantly reduced the production of major catabolic factors such as metalloproteases, IL-1β, and peroxynitrite, as well as COX-2 expression (24, 25). More recent studies have shown that cells from iNOS$^{-/-}$ animals had a marked reduction in prostaglandin E_2 (PGE_2) formation compared with cells from control animals (26). However, COX-2 protein expression was not significantly different in cells from control versus knockout animals. Additionally, levels of PGE_2 in the urine of iNOS-deficient mice were decreased by 78% compared with control animals. These studies support the hypothesis that NO and/or NO-derived species modulate COX activity and eicosanoid production in vivo.

A better understanding of the role of COX-2 in inflammation led to drug discovery programs aimed at identifying new anti-inflammatory agents that selectively inhibit COX-2 activity (27). It appears that COX-2 specific inhibitors are useful alternatives for the treatment of osteoarthritis and rheumatoid arthritis, particularly in patients at high risk of developing gastrointestinal complications.

Celecoxib (Celebrex) is a COX inhibitor that exhibits relative in vitro and in vivo selectivity for COX-2 over COX-1. Celecoxib was found superior to placebo and has similar efficacy to that of conventional NSAIDs in reducing the signs and symptoms of osteoarthritis and rheumatoid arthritis, as indicated by randomized, double-blind, multicenter studies (reviewed in 28). This drug reduced pain and inflammation for up to 24 weeks of treatment in clinical trials. Another placebo-controlled, randomized, double-blind, multicenter trial demonstrated that celecoxib was effective in treating osteoarthritis, as measured by clinical improvement in signs and symptoms comparable to results seen in patients on naproxen for symptomatic osteoarthritis of the knee (29).

Rofecoxib (Vioxx) is another COX-2 selective inhibitor approved for use in humans. Phase II and phase III clinical trials evaluating the efficacy of rofecoxib demonstrated that a dose of 25 or 50 mg once daily was effective and generally well-tolerated in patients with rheumatoid arthritis (30), and its clinical efficacy was comparable to that of a daily dose of 150 mg of diclofenac over a one-year study period (31). Additionally, both 12.5 and 50 mg of rofecoxib daily demonstrated clinical efficacy for treatment of osteoarthritis that was comparable to a high dose of ibuprofen (32).

COX-2 is thought to be involved in the inflammatory process. Inhibition of its activity achieves the same therapeutic effect provided by less specific inhibitors that also target COX-1, and offers superior gastrointestinal safety. Based on animal and clinical data, rofecoxib is now commercially available in the United States and United Kingdom for the treatment of pain and osteoarthritis, and celecoxib has been

approved in the United States and other countries for the treatment of rheumatoid arthritis and osteoarthritis.

PAIN Local tissue injury and inflammatory diseases such as osteoarthritis are associated with elevated PG production and increased sensitization of pain receptors to PGs (33). Thus, the action of COX at the site of injury or inflammation is hyperalgesic, and local pain relief following NSAID treatment is easily explained by this mechanism. In addition, PGs are thought to act in the spinal cord to facilitate the transmission of pain responses, though little is known about the mechanism for this effect. NSAIDs can also act centrally (34–36).

COX-2 is induced in both local and central sites (37), and the question of whether COX-2 mediates pain reception or transmission is currently being investigated, primarily through the use of COXIBs. Intrathecal injection of both the COX-2 selective inhibitor NS-398 and the nonselective NSAID indomethacin suppressed a formalin-mediated pain response (which measures a central response), but neither inhibitor suppressed a high-temperature-induced pain response (a local response) (38). In contrast, meloxicam, when given systemically, suppressed the inflammatory pain response locally (39) without affecting central pain transmission. Meloxicam, at low doses, is more selective for COX-2 than COX-1. In neither of these studies was the drug introduced into both sites to allow an internal comparison, but collectively this work shows that COX-2 can act both locally and centrally to mediate pain. Short-term human studies showed that celecoxib and rofecoxib effectively suppress the pain associated with dental extractions, osteoarthritis, or rheumatoid arthritis without causing any significant gastroduodenal toxicity (40–44). Additionally, rofecoxib has been found to be effective for treatment of primary dysmenorrhea (45).

Central Nervous System and Brain

PHYSIOLOGICAL FUNCTION OF COX-2 COX-2 appears to play some role in the regulation of brain function. PGs have long been known as mediators of fever, of inflammatory reactions in neural tissue, and, more recently, of brain function. The recognition that each of these processes involves induction of PG synthesis has led to an appreciation of COX-2's role in the PG-mediated functions. Although NSAIDs are commonly used to control fever, the actual mechanism of fever induction has only recently been elucidated. Intraperitoneal injection with lipopolysaccharide causes a marked fever response in rats. In an elegant dissection of molecular and tissue interactions, Cao and colleagues have shown that COX-2 induction in brain endothelial cells temporally parallels the fever response (46, 47). This leads to the synthesis of PGs, which then act on temperature-sensing neurons in the preoptic area. In turn, COX-2 inhibition by an isoform-specific NSAID can effectively block the fever response (48). Communication between local inflammatory sites and the brain endothelium is mediated by cytokines such as IL-1, which can directly induce COX-2 expression in these cells

(49). These investigators have also shown induction of COX-2 expression in other parts of the brain, but these areas are not directly associated with the fever pathway.

A separate inflammatory pathway is mediated by microglial cells, a type of tissue-specific macrophage that lies dormant until needed for defense or tissue remodeling (50). Though known as a source of PGs during inflammation, the microglial cells do not induce COX-2 in response to cytokines, unlike other inflammatory cells. Instead, the microglial COX-2 response is limited to direct lipopolysaccharide exposure, which would occur only by direct bacterial infection of the brain. Thus, the microglial defensive response is segregated from systemic inflammation by its limited repertoire of inducers.

Recent studies suggest involvement of COX-2 in amyotrophic lateral sclerosis (ALS), a neurodegenerative process. COX-2 inhibition may have some promise as therapy for the treatment of ALS (51, 52). Further studies are needed to explore this issue.

ALZHEIMER'S DISEASE The molecular and therapeutic mechanisms of Alzheimer's disease (AD) and inflammation have recently been reviewed (53–55). AD is characterized by progressive dementia and the extracellular deposition of β-amyloid fibrils within the brain. Subsequently, there is a phenotypic activation of microglial cells associated with the amyloid plaque. The amyloid-β peptide (Abeta) is a proteolytic fragment of the amyloid precursor protein (APP). Microglia activation results in a complex local proinflammatory response and secretion of inflammatory products.

Several epidemiological studies have indicated that patients taking NSAIDs for other diseases (e.g., rheumatoid arthritis) have a 50% lower risk of developing AD than those not taking NSAIDs (56–58). However, the precise pharmacological actions of anti-inflammatory drugs in the brain are still unclear. Several studies are attempting to identify a role for COX in the etiology of AD.

Cytokines such as IL-1 or IL-6, as well as acute-phase proteins such as α1-antichymotrypsin (ACT), participate in the etiopathology of AD. Tepoxalin, a novel NSAID, markedly inhibited IL-1β-induced IL-6 and ACT synthesis in astrocytes (59). Lipopolysaccharide-stimulated microglial cells treated with tepoxalin also exhibited decreased synthesis of IL-1β and IL-6 (59). This effect was mediated through inhibition of NF-κB via decreased IκB-α degradation. NF-κB is known to activate COX-2 expression under some circumstances. The β-amyloid-stimulated secretion of proinflammatory products by microglia and monocytes, mediating neurotoxicity and astrocyte activation, was also inhibited by NSAIDs, reportedly through $PPAR_\gamma$ activation (60).

Recognition of COX-2's key role in inflammation led to the hypothesis that it may represent a primary target for NSAIDs in AD, consistent with inflammatory processes occurring in AD brain (61, 62). Elevated CSF PGE_2 levels are observed in patients with probable AD (63). COX-2 was elevated in the hippocampal pyramidal layer in sporadic AD and was correlated with amyloid plaque density (64). In

vitro studies using COX-2–overexpressing neurons derived from transgenic mice suggest that elevation of COX-2 may potentiate A-beta–mediated oxidative stress (64). Further analyses of 54 post-mortem brain specimens from patients with normal or impaired cognitive status suggested that neuronal COX-2 expression in subsets of hippocampal pyramidal neurons may be a marker of progression of dementia in early AD (65). IL-1β and synthetic β-amyloid peptides induced COX-2 expression and PGE_2 release in the human neuroblastoma cell line SK-N-SH (66, 67). As demonstrated in human breast cancer cells (68), neuroblastoma cells also exhibit increased COX-2 expression mediated by p38 mitogen-activated protein kinase (MAPK), suggesting p38 MAPK as a potential therapeutic target in AD (67).

However, COX-1 and COX-2 may be involved in different cellular processes in the pathogenesis of AD, as indicated by their different distribution profile. An overall increase of COX-1 expression in AD has also been suggested. COX-1 expression was detected in microglial cells, whereas COX-2 expression was found in neuronal cells (69). In AD brains, COX-1-positive microglial cells were primarily associated with the amyloid plaques, and AD fusiform cortex exhibited increased density of COX-1 immunopositive microglia (69, 70). Furthermore, more COX-2-positive neurons were detected in AD brains than in control brains (69). Although in vitro studies use astrocytes to investigate the role of COX in AD, no COX expression was detected in astrocytes in vivo. Therefore, COX-1 could also contribute to central nervous system pathology, which brings up the issue of whether nonselective inhibitors would be more effective.

The possible implication of COX-1 in AD is further substantiated by the Alzheimer's Disease Cooperative Study (ADCS) (71). A multicenter clinical trial found that a repressor of COX-2 expression, prednisone, neither prevented nor accelerated cognitive decline in AD, although interpretation of these data is complex because glucocorticoids are fairly nonspecific and affect many other pathways. Nevertheless, the ADCS has initiated a trial to compare a nonselective NSAID and a selective COX-2 inhibitor for effectiveness in slowing the rate of cognitive decline in AD. Indomethacin showed promising results in a pilot clinical trial (72). Whether COX-2 inhibitors will be more effective is uncertain, since the enzyme is constitutively expressed in neurons and may play some role in normal brain function (73). Animal experiments suggest that COX-2 may be responsible for the regulation of adaptive functions associated with normal neurons and protective functions associated with stressed neurons. Other mechanisms for NSAID neuroprotective potency unrelated to their ability to inhibit COX-1 or COX-2, such as inhibition of monocyte cytotoxicity, have been suggested based on in vitro neurotoxicity assays (74).

The antithrombotic activity of PGs may also be important for protection against AD. For example, de la Torre (75) hypothesizes that AD is caused by the development of tortuous and flow-impeded capillaries in the brain. This would presumably promote intravascular coagulation, leading to ischemic damage in the brain that could promote the development of AD. Platelets contain both APP and

A-beta, which may contribute to the perivascular amyloid deposition seen in AD. Skowronski et al. (76) provide evidence that in human platelets, protein kinase C (PKC) is involved in the secretory cleavage of APP, whereas COX plays only a minor role in this process.

The precise role of the COX isoenzymes in AD is not clear, but the use of NSAIDs that inhibit both COX-1 and COX-2 activity appears to be beneficial. Nonselective NSAIDs can reduce inflammation associated with activation of microglia, but they seem ineffective in reversing the degenerative process in AD. Nevertheless, the effects of NSAIDs are likely to be mediated through a combination of mechanisms. Although reduced microglial or monocyte activation has been shown to be effective in various cell culture and animal models, clinical studies have yet to be performed. Mechanistic studies already under way will provide insight and direction for further developments.

Kidney

RENAL FUNCTION PGs are important physiologic modulators of vascular tone and salt and water homeostasis in the mammalian kidney. Their functions include modulation of glomerular hemodynamics, tubular reabsorption of salt and water, and regulation of renin secretion (77–79). While COX-1 has long been recognized to be involved in normal kidney function, COX-2 is thought to have a distinct role. Localization studies have found COX-2 in both the macula densa of the rat kidney (80) and the interstitial cells of the medulla (81). The macula densa plays an important role in mediating the interaction between glomerular filtration, proximal reabsorption, and regulation of renin release (82), which in turn is responsible for salt balance and fluid volume. Although PGE_2 has been reported to inhibit chloride reabsorption in the ascending limb of Henle, chronic salt deprivation was found to increase COX-2 levels in the region of the macula densa, and COX-2-generated prostanoids may be important mediators of renin production and tubuloglomerular feedback. The details of interactions between the COX-1- and COX-2-mediated systems in the kidney are not clear. Mapping of PG receptors in the kidney (83, 84) does show differential location of receptors specific for different PGs, indicating that differential synthesis of specific types of PGs may be responsible for the effects of COX-1 and COX-2. This topic has recently been reviewed (85).

In addition to the multiple roles of PGs in the adult kidney, the original strains of COX-2 null mice show severe disruption of kidney development (86, 87). Studies in COX-$2^{-/-}$ mice demonstrate that tissue-specific and time-dependent expression of COX-2 may be necessary for normal postnatal renal development and for maintenance of normal renal architecture and renal function (88). However, in later generations, the COX-$2^{-/-}$ mice demonstrate a much less severe phenotype with regard to renal function.

NSAIDs are known to have multiple effects on kidney function, and specific COX-2 inhibitors should be useful in dissecting the role of PGs generated from the

COX-2 pathway in normal renal physiology. However, caution is advised in clinical practice, since patients with chronic renal insufficiency taking COX-2 inhibitors may develop acute renal failure (89).

NEPHRITIS Biopsies of patients with IgA nephritis showed higher expression of COX-1, relative to COX-2, in glomeruli, whereas COX-2 was strongly expressed in infiltrating interstitial cells (90). Both COX isoforms may thus play a role in human glomerular inflammation associated with IgA nephritis.

In a rat model of transient mesangioproliferative glomerulonephritis, a dramatic transient increase of COX-1 staining in diseased glomeruli, localized mainly to mesangial cells, coincided with cell proliferation (90). A transient increase in COX-2 expression occurred in the macula densa region, and glomerular cells did not exhibit significant upregulation of COX-2 at any time. It was concluded that glomerular COX-1, but not COX-2, mediated PG production, which may contribute to the resolution rather than to the progression of nephritis in this rat model. In addition, regulatory interactions between the arachidonic and nitric oxide pathways in glomerulonephritis have been reported (91).

GASTROINTESTINAL TRACT

Maintenance of Gastrointestinal Integrity

The intestinal epithelium undergoes constant regeneration and remodeling in response to both insult and normal use. The use of NSAIDs can cause a variety of problems in the gastrointestinal tract (92), including irritation and ulceration of the stomach lining (93). Radiation exposure leads to intestinal epithelial cell death, leaving crypt cells to regenerate the epithelial lining. In animal studies, COX-2 is not induced following exposure to radiation and is not essential for crypt cell survival under these circumstances (94). Following radiation treatment, COX-1 plays a major role in maintaining proper glandular architecture of the small intestine and in maintaining healthy gastric mucosa. For example, indomethacin, which effectively inhibits COX-1 and COX-2, suppressed crypt survival and PGE_2 production in the intestine following radiation damage (94).

The gastrointestinal epithelium is also the target of numerous infectious and parasitic organisms. In response to infection or invasion, COX-2 expression is induced in epithelial cells (95), which leads to increased PG production. The PGs then stimulate chloride and fluid secretion from the mucosa, which flushes bacteria from the intestine. In addition, COX-2 is expressed during inflammation and wound healing, and in animals, treatment with COX-2 inhibitors can exacerbate inflammation and inhibit healing (96–98). Nevertheless, COX-2 selective inhibitors appear to be associated with less gastrointestinal damage than conventional NSAIDs (99). Clinical trials evaluating agents that are highly selective for COX-2 have demonstrated that selective COX-2 inhibitors have a significantly better safety profile than nonselective NSAIDs (100, 101).

Cancer

Several population-based studies have detected a 40%–50% decrease in relative risk for colorectal cancer in persons who regularly use aspirin and other NSAIDs (102–106). Studies in a variety of colon cancer animal models (both genetic and carcinogen-induced) have also demonstrated a significant reduction in tumor multiplicity following NSAID treatment (107). In fact, some studies have shown as much as an 80%–90% reduction in tumor burden (108).

Initial attempts to determine the molecular basis for these observations revealed that the majority of both human and animal colorectal tumors express high levels of COX-2, whereas the surrounding tissue has low to undetectable COX-2 expression (14, 109–111). Although COX-2 appears to play a role in colon cancer, the molecular mechanisms are only partially understood. Processes recently recognized as important include the inhibition of tumor cell growth, prevention of angiogenesis, and induction of apoptosis in neoplastic cells (Figure 2).

Celecoxib has been shown to dramatically inhibit colon carcinoma growth in preclinical studies, both in vitro and in vivo, without toxicity to the gastrointestinal tract (112). These results support the need for additional clinical studies of celecoxib for treatment and/or prevention of colorectal cancer in humans. Other work in cell culture models has shown that COX-2 expression contributes significantly to the tumorigenic potential of epithelial cells by increasing adhesion to extracellular matrix and making cells resistant to apoptosis (113). These phenotypic changes are reversed by treatment with a highly selective COX-2 inhibitor. Immunohistochemistry and RT-PCR measurements of COX-2 in sporadic colorectal cancers, including adenomas, carcinomas, hyperplastic lesions, and normal tissues suggested that enhanced expression of COX-2 occurs early during colorectal carcinogenesis and may contribute to tumor progression (114). In this regard, a number of *cis* regulatory elements are present within the COX-2 promoter that may be involved in its upregulation during progression to neoplasia (Table 2).

TABLE 2 Identified regulators of COX-1 and COX-2 gene expression

COX-1	**COX-2**		
Upregulators	**Upregulators**		**Downregulators**
iNOS	iNOS	EGF	p53
Estrogen	IL-1α	TGF-β	Fish oil
	Wnt-1	TNF-α	Antioxidants
	Wnt-3	UVB	
	Src	Estrogen	
	Ras	Androgen	
	Benzo[α] Pyrene		

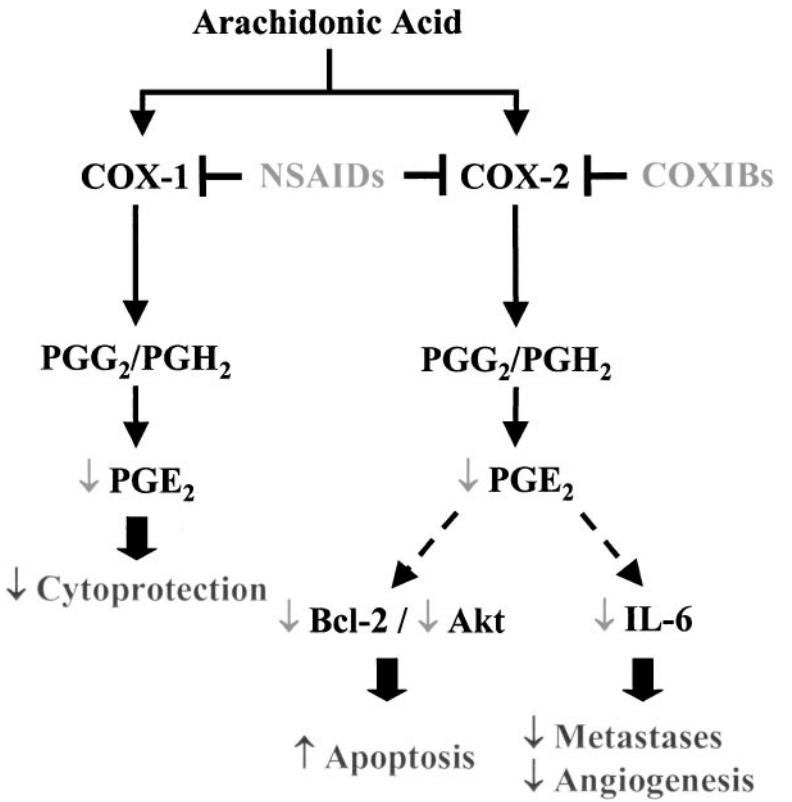

Figure 2 Potential mechanism of COX-2 inhibitors in neoplasia. COX-2 produces prostaglandins that inhibit apoptosis and stimulate angiogenesis and invasion. Prostaglandin synthesis can be reduced by selective COX-2 inhibitors to restore apoptosis and inhibit cancer cell proliferation. These effects may be mediated through the inhibition of IL-6 production and the downregulation of Bcl-2 and Akt.

Several studies indicate that COX-independent pathways are also important in the cancer chemopreventive properties of NSAIDs, and it is likely that both COX-dependent and COX-independent effects are involved (115–117). For example, certain NSAIDs induce apoptosis and alter expression of cell cycle regulatory genes in some cell lines when administered at relatively high concentrations (200–1000 μM) (115, 118). By using COX-deficient cell lines or drug metabolites lacking COX-inhibitory activity, these studies rule out the involvement of COX in the growth-inhibitory effect (112). Certainly, this class of drugs can affect biochemical pathways unrelated to COX, and these effects appear to be dose-dependent (some effects occurring only at toxic doses). He et al. (119) have implicated a direct effect of sulindac (another NSAID) on inhibition of $PPAR_{\delta}$-directed transcription in cell culture models, but only at drug concentrations above the 100-μM range.

More recently, this group has shown that sulindac has similar effects on cells that completely lack the $PPAR_{\delta}$ gene (120), indicating that other targets are probably responsible for this effect. The specific mechanisms of these COX-independent effects and their therapeutic implications are not yet well understood. However, most of the studies demonstrating effects on COX-independent pathways utilize concentrations of NSAID (100–1000 μM) that are difficult to achieve in living organisms without severe toxic side effects.

Familial Adenomatous Polyposis

Clinical trials with NSAIDs in patients with familial adenomatous polyposis (FAP) have clearly demonstrated that NSAID treatment results in regression of preexisting adenomas (121). Genetic evidence supporting a role for COX-2 in the development of intestinal neoplasia has also been reported. Oshima et al. (122) assessed the development of intestinal adenomas in $Apc^{\Delta 716}$ mice (a model in which a targeted truncation deletion in the tumor suppresser gene APC causes intestinal polyposis) in a wild-type and homozygous null COX-2 genetic background. The number and size of polyps were reduced six- to eight-fold in the COX-2 null mice compared with COX-2 wild-type mice. In addition, a COX-2 inhibitor, Merck Frosst (MF) tricyclic, reduced polyp number in the $Apc^{\Delta 716}$ mice more significantly than the nonselective NSAID, sulindac (122). Jacoby et al. (123) provided further support for a role of COX-2 by demonstrating that celecoxib was effective for the prevention and regression of adenomas in the adenomatous polyposis coli (APC) mutant Min mouse model. These and other studies (124) support ongoing clinical trials of COX-2 selective inhibitors in humans with FAP. Treatment twice daily for six months with celecoxib (400 mg) resulted in a significant reduction in the number of colorectal polyps in patients with FAP (125), leading to U.S. Food and Drug Administration (FDA) provisional approval of this drug for use in FAP patients.

Angiogenesis

Angiogenesis, the formation of new capillaries, is essential not only for the growth and metastasis of solid tumors but also for wound and ulcer healing. Blood flow for oxygen and nutrient delivery to the healing site cannot be restored without angiogenesis. Angiogenesis and suppressed cell-mediated immunity are central to the development and progression of malignant disease (reviewed in 126). Recent work indicates that COX may play a very important role in the regulation of angiogenesis associated with neoplastic tumor cells (127).

NSAIDs, such as aspirin, have antiangiogenic and immunomodulatory properties. COX-2 contributes to tumor angiogenesis through various mechanisms (reviewed in 128). Key mechanisms appear to involve the increased expression of the proangiogenic growth factor VEGF (129); the production of the eicosanoid products thromboxane (TX) A_2 (130), PGE_2, and PGI_2, which can directly stimulate endothelial cell migration and growth factor–induced angiogenesis; and,

potentially, the inhibition of endothelial cell apoptosis by induction of Bcl-2 expression or Akt activation.

Both selective and nonselective NSAIDs inhibit angiogenesis through direct effects on endothelial cells (131). This effect is mediated through inhibition of MAPK (ERK2) activity and interference with ERK nuclear translocation but is independent of protein kinase C. It also involves prostaglandin-independent and prostaglandin-dependent components. In some circumstances, both COX-1 and COX-2 appear to be regulators of angiogenesis (132).

Other Cancers

Overexpression of COX-2 may not be unique to colon cancer and does occur in other epithelial tumors. Elevated COX-2 expression was reported in human breast cancers (133), lung cancer (134), uterine carcinoma (135), and carcinoma of the cervix (136). In vitro and animal experiments also suggest a role of COX-2 in bladder cancer (137, 138) and skin cancer (139, 140). A possible therapeutic effect of COX inhibition has also been suggested for head and neck cancers (141, 142) and esophageal cancer (143, 144).

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is known to be associated with increased local production of prostanoids (145). Chronic intestinal inflammation (especially long-standing pancolitis) is directly linked to an increased lifetime risk for colorectal cancer.

Animal studies investigating the role of COX-2 in IBD have yielded conflicting results. Karmeli et al. (146) reported beneficial effects of COX-2 inhibitors on the extent and severity of experimental colitis in two rat models. Colitis was induced by intracaecal administration of 2 ml 5% acetic acid or intracolonic administration of 0.1 ml 3% iodoacetamide. On the other hand, three highly selective COX-2 inhibitors, NS-398, SC-58125, and PD-138387, did not exhibit any beneficial effect in the trinitro-benzene sulfonic acid (TNBS) model of colitis in rats (147). In agreement with the latter study is the report of PGD_2-mediated downregulation of granulocyte infiltration into the colonic mucosa, probably through the DP receptor in the same TNBS model of colitis (148). The increase in PGD_2 synthesis was abolished by treatment with a selective COX-2 inhibitor and resulted in a concomitant doubling of granulocyte infiltration. On the other hand, aspirin, a COX-1–preferential inhibitor, was more effective than selective COX-2 inhibitors at inhibiting granuloma dry weight, vascularity, and COX activity in the murine chronic granulomatous tissue air-pouch model of chronic inflammation (149).

In IBD patients, a relationship between endoscopic activity and relative levels of COX-2 mRNA has been reported (150). Whereas COX-2 was undetectable in normal ileum or colon, it was induced in apical epithelial cells of inflamed foci and in mononuclear cells of the colonic lamina propria of biopsies from IBD patients (151). COX-1 expression in inflamed tissue was similar to that of normal tissue

(150–152). Differences in the effects of inhibitors in experimental colitis may be due to differences in the animal models and in the COX inhibitors used. However, the ability of NSAIDs and COX-2 inhibitors to exacerbate IBD (148, 153) suggests that PGs are important anti-inflammatory mediators in this context, or that COX inhibition results in shunting of the arachidonic acid substrate to other pathways such as lipoxygenase for production of proinflammatory leukotrienes (e.g. LTB_4). Whether inhibition of COX-2 would improve symptoms in patients suffering from chronic IBD is presently unclear. To our knowledge, no studies evaluating the effect of COXIBs in IBD patients have been reported.

CONCLUSIONS

The COX isoenzymes and their eicosanoid products play functional roles in many physiological systems. NSAIDs such as aspirin, indomethacin, and ibuprofen are the most widely used drugs for pain, arthritis, and cardiovascular diseases and now are under consideration for the prevention of colon cancer and AD. COX-2 selective agents appear to be an improvement over conventional NSAIDs for patients with pain, rheumatoid arthritis, and osteoarthritis, which has resulted in their widespread use in medical practice.

The ability of NSAIDs to exacerbate IBD in both humans and animals suggests that prostanoids are important anti-inflammatory mediators in this context. In addition, specific COX-2 inhibitors have been reported to exacerbate chronic inflammation in animals. Because of the adverse effects reported in animal studies, a trial testing the efficacy of COX-2 inhibitors in IBD patients is unlikely.

Constitutive COX-2 expression has been detected in the stomach, kidney, pancreatic islet cells, and central nervous system, suggesting a homeostatic role for COX-2 in certain tissues. In addition, both COX isoenzymes play an important role in tissue repair. The safety of COX-2 inhibitors in patients with active ulcers or with cardiovascular or renal disease requires further investigation.

Arachidonic acid metabolism through the COX and lipoxygenase pathways generates an array of bioactive eicosanoids. The mechanisms by which this biosynthetic pathway can mediate such diverse functions are largely unknown and likely to remain so until the various PG synthases and receptors downstream of COX are more fully characterized. The production of leukotrienes and their role in inflammation and cancer should not be overlooked.

Advancements in NSAID research have enabled the development of the COXIBs, a new class of NSAIDs that have quickly moved into clinical use. The quest for new drugs may lead to the development of additional compounds targeted toward specific eicosanoids (such as TXA_2), their synthetic enzymes, or their specific receptors to allow for the normal physiologic effect of eicosanoids without a pathologic response. Specific pathways, downstream of the COX/lipoxygenase enzymes involved in pathogenesis, theorically could be modulated with minimal alterations in the production of eicosanoids necessary to maintain homeostasis. This may result in more effective therapies for an array of diseases.

A little more than a century after the discovery of aspirin, the potential clinical indications for NSAIDs are widening from their original use as analgesics. Ongoing studies to more clearly delineate the role of each COX isoform in both health and disease will be crucial in defining additional applications for these drugs in the next century and in determining their ultimate safety.

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