Cyclooxygenases

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Key Concepts

- *Cyclooxygenase (COX) enzymes catalyze the rate limiting steps in prostaglandin synthesis. Prostaglandins play major roles in diverse physiological processes such as maintenance of GI mucosal integrity and pathological processes such as inflammation and neoplasia.*

- *Two COX isoforms, COX-1 and COX-2 exist in higher organisms. They are highly similar in structure and enzymatic activity. The main differences between the two lie in their genetic regulation and biological roles. COX-1 expression is constitutive in most cell types and is thought to carry out "housekeeping" roles in the various tissues. In contrast, COX-2 expression is induced in response to inflammatory and proliferative stimuli.*

- *COX enzymatic activity is the target of widely used non-steroidal anti-inflammatory drugs NSAIDs). COX-2-selective NSAIDs are currently being developed in the hopes of limiting inflammation without adverse GI and renal effects.*

- *The cellular source of inducible COX-2 activity in acute and chronic GI inflammation and neoplasia is currently poorly understood.*
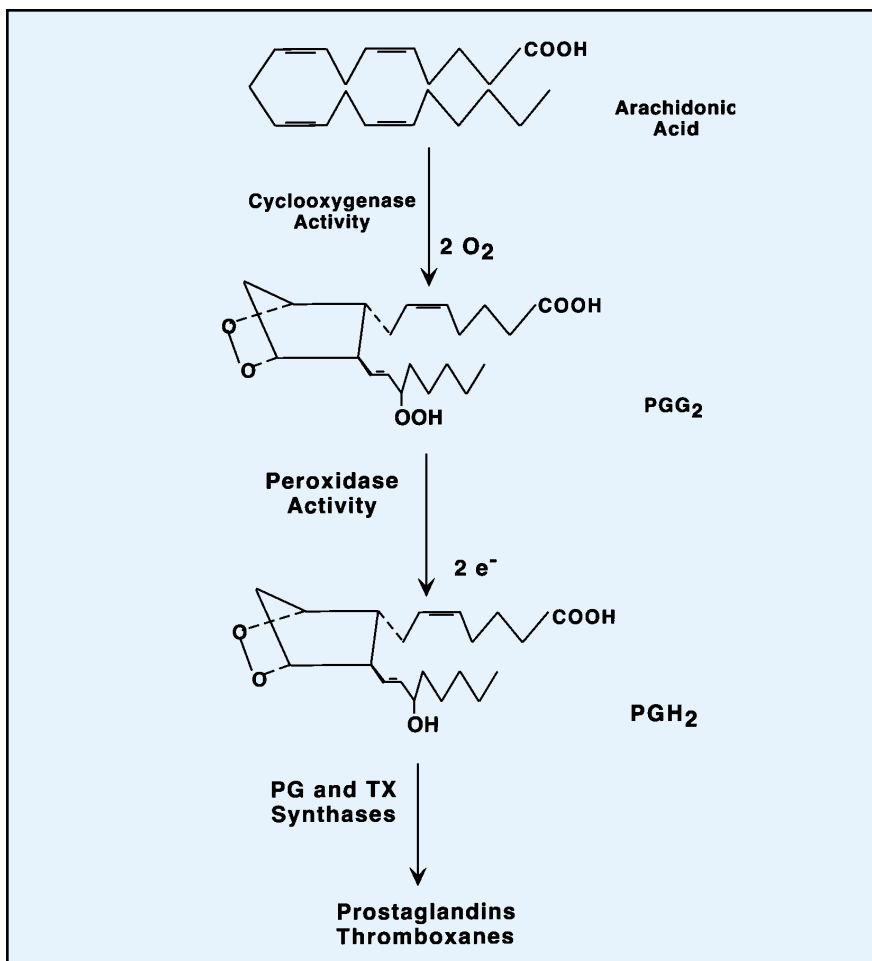

Figure 1. Prostaglandin Biosynthesis.

The Cyclooxygenase Reaction

Cyclooxygenase (COX) enzymes, also referrred to as prostaglandin H synthases or prostaglandin endoperoxide synthases, catalyze the rate limiting steps in prostaglandin (PG) and thromboxane (TX) synthesis (Fig. 1). Enzymatic COX substrates are 20 carbon polyunsaturated fatty acids, most often

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Introduction

Don W. Powell, M.D.
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The development of clinically effective inhibitors specific for cyclooxygenase 2 (Cox 2) is a dramatic example of the power of modern biological research at the turn of the 20th Century. In less than ten years after this second isoform of cyclooxygenase was cloned, our understanding of the formation of prostaglandins, their roles in normal biology and disease, and potential ways to inhibit their formation have been revolutionized as a result of studies of the molecular biology and structure of these enzymes. This is a prime example of the application of molecular biology and structural biology to drug development.

The first article in this issue of the *Regulatory Peptide Letter* by R.C. Mifflin and D.W. Powell summarizes the biology of the two cyclooxygenase enzymes. The authors briefly describe the biochemistry of prostaglandin (PG) formation, the known functions of the many members of the PG family, and the various distinct receptors through which the PGs have their biologic effects. They review the molecular and structural organization of the Cox isoforms and how the molecules that make up the enzyme determine its structure. This structure has allowed industry to create specific inhibitors for the Cox 2 isoform. In the last part of the article, the authors also review less well-known areas of cyclooxygenase biology such as the regulation of Cox gene expression in gastrointestinal tissues. The latter is important because an inhibition of cyclooxygenase in the stomach and intestine leads to gastrointestinal damage; side effects which limit the clinical effectiveness of cyclooxygenase inhibitors.

The second article by Dr. Mark Feldman reviews the role of cyclooxygenase in maintaining the barrier function of the gastrointestinal tract. Dr. Feldman reviews the idea of the "cytoprotective effect" of prostaglandins in the gastrointestinal mucosa. He lists the evidence for the concept that cyclooxygenase 1 is the "housekeeping" form of the prostaglandin-forming enzymes that maintains structure and basic function in tissues, whereas Cox 2 is the isoform that produces prostaglandins during inflammatory states. The prostaglandins are the cause of pain, erythema, warmth and the edema of inflammation. These proposed differences in the two Cox isoforms have led to the concept of "good" cyclooxygenase (Cox 1) and "bad" cyclooxygenase (Cox 2). He reviews the Cox 2 therapeutic hypothesis, which says that highly selective Cox 2 inhibitors should be efficacious in inflammatory disease without causing the severe side effects, particularly GI toxicity, which limits the usefulness of this class of drugs. Dr. Feldman points out that although early reports of the clinical trials of the specific Cox 2 inhibitors now approved in the United States, Celecoxib® and Rofecoxib®, tend to support the concept of a Cox 2 therapeutic hypothesis, it is still early in the use of these drugs. Their ultimate efficacy and side effect profile will require post-marketing studies of the millions of patients with inflammatory diseases who use the drugs.

In the last article, Drs. R.N. DuBois and M. Mann review the concept that inhibition of cyclooxygenase enzymes has a therapeutic role in cancer risk reduction in the gastrointestinal tract, especially for human colorectal carcinoma. The authors review the role of Cox inhibition in the prevention of sporadic colorectal cancer and their therapeutic effect in reducing the size and number of colonic adenomas in a genetic form of colon cancer, familial adenomatous polyposis (FAP). These authors also review the potential mechanisms for the chemopreventive effect of nonsteroidal anti-inflammatory drugs (NSAIDs) in the prevention of colorectal cancer. Studies in animals suggest that the chemopreventive effects of NSAIDs may occur through specific inhibition of Cox 2 enzymes. If this is the case, then Cox 2 specific inhibitors might have a positive chemopreventive effect without the gastrointestinal side effects of Cox 1 inhibition. While it is too early to be sure if this variation of the Cox 2 therapeutic hypothesis is correct, certainly it is an exciting idea that will result in much basic and clinical investigation in the coming few years.

The cloning of a second isoform of the cyclooxygenase enzyme, as well as molecular and structural studies of both proteins, have led to a new class of therapeutic agents in less than a decade. While the ultimate promise and efficacy of these drugs remains to be determined through their extensive use in humans with disease, the creation of specific Cox 2 inhibitors represents an important paradigm for the development of pharmacologic agents in the 21st Century. ◼

arachidonic acid (AA), released from phospholipid of cellular membranes by a phospholipase A_2. The first step involves addition of O_2 atoms to C-11 and C-15 to yield PGG_2. Since this results in cyclization of the fatty acid this is referrred to as the cyclooxygenase activity. The 15-hydroperoxide group of PGG_2 is then converted to an alcohol forming PGH_2 by the peroxidase activity of the enzyme. PGH_2 is subsequently converted to other PGs (PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2) or thromboxanes (TXA_2) by specific cellular synthases (Fig. 1).

Functions of Prostaglandins

PGs play critical roles in normal physiological processes. Platelet-derived TXA_2 is an important mediator of platelet aggregation and thus hemostasis. During periods of stress, PGs of the E and I series are important regulators of renal blood flow. PGs likewise are important in modulating many aspects of reproductive biology including ovulation, fertilization, fetal development, and parturition. The opposing actions of different PG classes help to maintain bronchial tone. The processes of bone formation and resorption are also subject to regulation by PGs. Macrophage differentiation is likewise modulated by PGs. As discussed in Dr. Feldman's article to follow, PGs are vital to the maintenance of mucosal integrity in the GI tract and also play a role in the regulation of motility and secretion. PGs also affect immune function in a number of ways. Through its ability to differentially inhibit cytokine synthesis by TH_1 cells, PGE_2 can shift the balance of an immune response in favor of TH_2 cells. PGE_2 also synergizes with IL-4 to activate

isotype switching to IgG1 and IgE in B cells. Important roles for PGs in CNS function and development have also been identified. For example, increased PGE_2 synthesis by hypothalamic endothelial cells is involved in the febrile response and PGs generated at sensory nerve terminals cause hyperalgesia. Increased PG synthesis has also been correlated with seizure activity.

PGs have been implicated in a wide variety of disease processes. The huge annual market for nonsteroidal antiinflammatory drugs (NSAIDs) which inhibit COX activity is a testament to the role of PGs in acute inflammation and chronic inflammatory diseases such as asthma, rheumatoid/osteo arthritis, and inflammatory bowel disease (IBD). Epidemiological and animal studies indicate that inhibition of PG synthesis is efficacious in the prevention of coronary artery thrombosis, Alzheimer's disease, and gastrointestinal and breast cancer. The properties of PGs that contribute to disease progression include their thrombotic activity, ability to modulate cellular apoptosis and other cell cycle parameters, angiogenic activity, and other functions yet to be identified. In the accompanying article, Dr. Dubois will cover the role of COX enzymes and PGs in the development of colorectal cancer and discusses their role in neoplasia in more detail.

PG Receptors

The effects of PGs upon cells are realized when each binds its specific membrane-bound receptor (Table 1). These constitute a homologous family of G protein-coupled receptors containing seven transmembrane domains. Differential responses to specific PGs are determined by the type of

Table 1. Membrane-Based Eicosanoid Receptors.
(Adapted from E.J. Goetzl, S. An, and W.L. Smith *FASEB J.* 9: 1051–1058, 1995.)

Eicosanoid	Receptor Name	Tissue Distribution*	Transductional Signal	Effects
PGE2	EP1	Smooth Muscle, Fibroblasts	Ca^{++} Mobilizatiion	SM Contraction Proliferation
PGE2	EP2	Smooth Muscle, Epithelial Cells, Mast Cells, Neurons, Fibroblasts	cAMP Increase	SM Relaxation, Stimulate Intestinal Secretion, Sensation, Inhibit Mast Cell Degranulation
PGE2	EP3	Smooth Muscle, Adipocytes, Neurons, Epithelial Cells. Kidney	cAMP Decrease Ca^{++} Mobilization	SM Contraction, Inhibit Lipolysis, Neurotransmitter Release, Stimulate Renal H_2O Reabsorption
PGE2	EP4	Fibroblasts, Myofibroblasts, Smooth Muscle	cAMP Increase	SM relaxation, Induction of Stellate Morphology in Myofibroblasts
PGD	DP	Platelets, Smooth Muscle, Neurons	cAMP Increase	SM Relaxation, Inhibition of Neurotransmitter Release, Platelet Aggregation
PGI	IP	Platelets, Smooth Muscle, Neurons	cAMP Increase	SM Relaxation, Inhibition of Platelet Aggregation, Stimulate Intestinal Secretion
PGF2α	FP	Kidney, Myofibroblasts, Astrocytes, Smooth Muscle	Ca^{++} Mobilization	SM Contraction, Myofibroblast Contraction
TXA2	TP	Platelets, Smooth Muscle	Ca^{++} Mobilization	SM Contraction, Platelet Aggregation, Glomerular Filtration, Intestinal Secretion

* not comprehensive

G protein ($G_{\alpha s}$, $G_{\alpha i}$, $G_{\alpha q}$, $G_{\alpha 12}$) coupled to each receptor. At least four distinct PGE_2 receptors exist which couple to different signaling pathways. As a general rule, $G_{\alpha s}$-coupled receptors result in increased levels of intracellular cyclic adenosine monophosphate (cAMP); $G_{\alpha i}$-coupled receptors result in inhibition of cAMP generation; and $G_{\alpha q}$-coupled receptors result in intracellular Ca^{2+} mobilization. Signaling via $G_{\alpha 12}$-coupled receptors is not completely undersood. Further diversity in the response to each PG is achieved through alternate splicing of PG receptor mRNAs generating different carboxyl termini.

Recent evidence also indicates that certain PGs, such as PGJ_2 and its derivatives, are also potent ligands for a class of receptors termed peroxisome proliferator-associated receptors (PPARs). These receptors are members of the nuclear hormone receptor superfamily of ligand activated transcription factors which target to the nucleus upon ligand binding. Thus certain PGs are able to directly modulate transcription of specific genes via interaction with PPARs.

COX-1 and COX-2

Two distinct COX enzymes exist. COX-1 was first purified and characterized in the 1970s and the gene was isolated in 1988. The discovery and cloning of the second COX isoenzyme, COX-2, in 1991 initiated a revolution in our understanding of PGs and their functions in normal physiology and disease.

The two enzymes are highly similar in structure and enzymatic activity. Both are homodimeric heme-containing proteins with a molecular weight of roughly 71 KDa. They share 63% identity at the amino acid level. COX-2 contains an 18 amino acid insertion in its carboxyl terminal region while COX-1 contains an 8 amino acid insertion at the amino terminus of the mature protein. Both proteins are glycosylated; three conserved N-linked glycosylation sites exist in both enzymes and COX-2 contains an additional site within the 18 amino acid insertion.

The mature proteins contain three distinct domains. The first is a conformation which is highly similar to that of epidermal growth factor (EGF) and is termed the EGF-like domain. The function of this domain in COX enzymes is poorly understood but is thought to facilitate recruitment and interaction with other cellular

proteins. The second domain contains a series of amphipathic helices which comprise the membrane attachment site. COX enzymes are unlike other integral membrane proteins in that they are not anchored via transmembrane domains. Instead, they associate with the endoplasmic reticulum (ER) membrane via hydrophobic interactions and are thus monotopic membrane proteins. It is interesting that while both enzymes are associated with the luminal face of the ER, COX-2 is also enriched in the perinuclear region. The association of COX-2 with the nucleus raises questions about a direct role of COX-2-derived PGs on gene expression via association with PPARs. The third domain is a large globular region which contains the cyclooxygenase and peroxidase active sites. The COX active site lies in a narrow hydrophobic channel framed by the membrane attachment helices which allows arachidonic acid cleaved by PLA_2 direct access from the ER membrane without having to transit a hydrophilic environment (Fig. 2). The amino acids involved in substrate binding and catalysis are by and large identical between the two enzymes. Two important differences are found at residues 434 and 523 (COX-1 numbering) where isoleucine occupies each position in COX-1 and valine is present in each position in COX-2. These amino acids are part of the substrate binding channel and one consequence of these substitutions is that COX-2 has a wider channel. This channel difference is the basis behind the broader substrate specificity of COX-2 and, as discussed below, the basis behind the design of drugs specifically targeted to inhibit COX-2. In fact, changing isoleucine 523 in COX-1 to valine renders it sensitive to some COX-2-selective inhibitors. Other amino acid
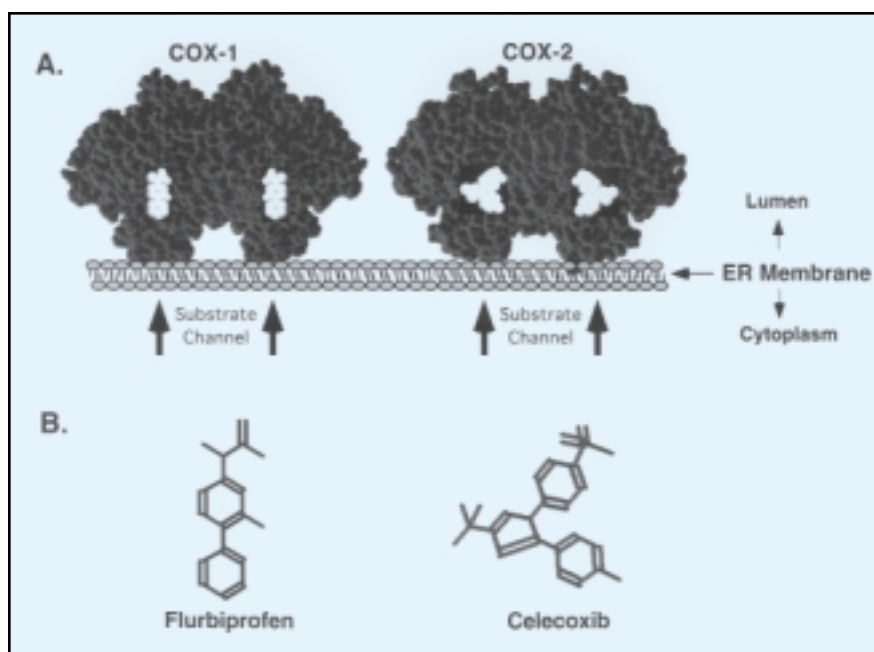

Figure 2. A: Diagrammatic representation of the orientation of COX-1 and COX-2 in the ER membrane. Shown are a COX-1 and a COX-2 homodimer demonstrating the association with the lumenal ER surface via the amphipathic helices. In the central portion of each monomer is shown a cutout section demonstrating NSAID binding to the active site. In the case of COX, the active site is narrower allowing access only to NSAIDs with smaller side chains. COX-2 specific NSAIDs contain larger side chains not accommodated by the COX-1 pocket. **B:** Diagrammatic representation of the molecular structure of flurbiprofen, a nonselective NSAID, and celecoxib, a COX-2 specific NSAID.

differences in this channel that also play a role in determining substrate and inhibitor specificity include histidine (COX-1) to arginine (COX-2) at position 513, and serine (COX-1) to alanine (COX-2) at position 516.

Another interesting difference between COX-1 and COX-2 is that each enzyme apparently utilizes a distinct source of cellular arachidonate as substrate resulting in a functional compartmentalization of COX-1 versus COX-2 activity. For example, aggregation of IgE receptors on mast cells results in a biphasic release of PGD_2. The first phase is mediated by COX-1 utilizing arachidonate released by a form of phospholipase A_2 called secretory PLA_2 while the second phase is mediated by COX-2 utilizing arachidonate released by a different phospholipase A_2 termed cytosolic PLA_2.

Mechanisms of COX Inhibition

Based upon their inhibitory mechanisms COX inhibitors can be grouped into four classes (Table 2). All but the first class are reversible inhibitors in that once the drug is removed, COX activity is restored, albeit at different rates depending upon the compound. The first class, which includes aspirin and recently developed COX-2-specific aspirin-like molecules irreversibly inactivate COX activity by acetylating an active site serine. Aspirin is considered COX-1 selective since doses 10 to 100 fold higher than those required for COX-1 are necessary to acetylate the COX-2 active site. Although aspirin-acetylated COX-1 retains no enzymatic activity, due to its larger substrate binding channel, acetylated-COX-2 retains its peroxidase activity, and is effec-

Table 2. Four Modes of COX Inhibition by NSAIDs.

Mode of Inhibition	Selectivity	Examples	Comments
Covalent Modification	COX-1 COX-2 (APHS)	Aspirin APHS*	Acetylation of active site serine
Reversible, Competitive Inhibition	COX-1 and 2	Ibuprofen Mefenamate	Compete with AA† for active site.
Slow, Time-dependent Inhibition	COX-1 and 2	Indomethacin Flurbiprofen	Salt bridge formation with Arg. 120
Time-dependent COX-2 Inhibition	COX-2	Celecoxib Rofecoxib SC58125	Larger side groups to occupy extra side pocket in COX-2

• o-(acetoxyphenyl)hept-2-ynyl sulfide
† arachidonic acid

tively converted into a lipoxygenase enzyme capable of generating 15-R-hydroxyeicosatetraenoic acid (HETE). Recently, the aspirin derivative, o-(acetoxyphenyl)-hept-2-ynyl sulfide (APHS) has been developed which exhibits increased selectivity and potency toward COX-2. APHS, like aspirin, irreversibly inactivates the cyclooxygenase activity of COX-2. APHS-modified COX-2 retains the ability to generate 15-R-HETE. The development of APHS as a COX-2-selective agent was based upon structural data of the COX-2 substrate channel and the observation that effective COX-2-selective inhibitors have sulfur-containing side chains in place of a carboxylic acidic group. APHS represents a parent compound which is certain to be followed by more effective, irreversible, COX-2 specific NSAIDs.

The second class of inhibitors consists of reversible, competitive inhibitors of both enzymes. These compounds compete with arachidonic acid for binding to the cyclooxygenase active site. Ibuprofen and mefenamate are examples of this class of inhibitor. Indomethacin and flurbiprofen exemplify the third class of COX inhibitor. These agents exhibit a slow, time-dependent inhibition of both COX isoforms. The delayed kinetics of inhibition by this class probably reflects the time necessary for formation of a salt bridge between the carboxylate of the drug and arginine 120 (COX-1 numbering).

The fourth class of COX inhibitors selectively inhibit COX-2. These include recently-developed drugs such as celecoxib and SC58125 which incoporate sulphonamide or sulphone groups in place of carboxylic acid. They also contain larger side groups which penetrate the larger binding pocket of COX-2, but their size prevents them from entering the smaller pocket of COX-1. These compounds are effective time-dependent inhibitors of COX-2; the time dependence is thought to reflect the time required for optimal insertion of the inhibitor into the deeper pocket of COX-2 (Table 2).

Regulation of COX Gene Expression

COX-1 expression is constant (constitutive) in most tissues and cell types. COX-1 is, therefore, considered as the isoform responsible for generation of PGs which mediate homeostatic or "housekeeping" functions such as maintenance of vascular tone and mucosal integrity in the GI tract. The human COX-1 promoter region resembles that of other housekeeping genes in that it lacks a TATA box and is generally not subject to transcriptional induction. However, COX-1 expression is subject to developmental and inducible regulation under certain circumstances. For example, stem cell factor (SCF) treatment of immature murine bone marrow derived mast cells results in a 6–8 fold induction of COX-1 mRNA and protein levels. Differentiation inducing stimuli (eg. transforming growth factor-β, phorbol esters) have also been shown to result in transient 1.5 to 3-fold induction of COX-1 expression in monocytes and macrophages. Estrogen-induced expression of COX-1 is responsible for the increase in PGI_2 synthesis in perinatal pulmonary vascular beds and is partly responsible for the pulmonary vasodilation seen during this period.

In contrast COX-2 expression is undetectable in most normal tissues. Important exceptions to this rule are the brain and renal cortex where constitutive COX-2 expression occurs. COX-2 expression in many cell types is highly induced in response to proinflammatory stimuli such as IL-1, TNF_α, and bacterial lipopolysaccharide (LPS). Nucleotide sequence analysis of the human COX-2 gene promoter reveals the presence of potential binding sites for a variety of transcription factors activated by inflammatory and proliferative stimuli. These include NF-1, AP-2, STATs, NFkB, NFIL6/cEBP, CREB/ATF, and E-box-binding proteins. Signaling pathways which play a role in COX-2 induction include

generation of cAMP, activation of protein kinase C isoforms, generation of inositol trisphosphates, generation of ceramide, activation of mitogen activated protein kinases (MAPKs) such as c-Jun N-terminal kinase (JNK), P38 kinase, and extracellular signal regulated kinases (ERKs), as well as Janus-associated kinases (JAKs). COX-2 gene expression is also subject to negative regulation. The anti-inflammatory cytokines IL-4, IL-10, and IL-13, and corticosteroids inhibit COX-2 expression. COX-2 expression is also regulated at post-transcriptional levels by various mechanisms including mRNA splicing, message stability, and translation. The 3′ untranslated region of the COX-2 mRNA contains multiple copies of the pentanucleotide motif AUUUA which confers message instability upon a number of cytokine and proto-oncogene mRNAs. Such motifs represent potential targets by which agents such as IL-1 stabilize, and corticosteroids destabilize the COX-2 message, thus promoting elevated or decreased levels of enzymatic activity, respectively.

COX Expression in GI Tissues

Numerous studies have documented expression of COX-1 throughout the length of the GI tract. COX-1 immunoreactivity has been demonstrated in crypt epithelial cells, endothelial cells of blood vessels, lamina propria mast cells, macrophages, lymphocytes, fibroblasts, and smooth muscle cells to name a few. Using sensitive detection methods, COX-2 mRNA can be found in normal stomach and intestinal tissue and occasional COX-2 immunoreactive inflammatory cells are seen. However, dramatic elevations in COX-2 expression occur in response to acute or chronic mucosal inflammation and ulceration.

The cell types in which COX-2 expression increases in response to mucosal injury and inflammation have not been clearly identified and defined. Epithelial cell COX-2 expression has been demonstrated following invasion by bacteria, in patients suffering from IBD, and at the latter stages of carcinogenesis. However, studies from several laboratories show that the vast majority of intestinal PG production in inflammatory conditions occurs in the lamina propria and submucosa. In a rat model of colitis, increased levels of COX-2 mRNA are seen and the bulk of immunoreactive COX-2 is localized to cells of the lamina propria in regions occupied by subepithelial myofibroblasts, mast cells, neutrophils, and smooth muscle cells, and in the muscularis of the colon. Likewise, recent studies using rat models of NSAID-induced gastric ulceration localized COX-2 expression to the lamina propria of regenerative regions. Interestingly, in a murine model of familial adenomatous polyposis coli, Oshima et. al. localized COX-2 transcription in early adenomas, not in epithelial cells, but to a location directly subjacent to the epithelial cells in the area occupied by intestinal subepithelial myofibroblasts.

The notion that COX-1 represents the "good COX" and COX-2 represents the "bad COX" is probably an oversimplification. Prolonged COX-1 inhibition certainly can produce adverse GI side effects (ulcers) while recently developed COX-2-specific inhibitors (see below) result in fewer ulcers. Furthermore, COX-2 inhibition may prove to be beneficial for chemoprevention of certain GI cancers (see accompanying article by Dr. Dubois). However, recent studies indicate that COX-2-derived PGs play a beneficial role in the healing of gastric and intestinal ulcers, and thus COX-2 inhibition in patients with already existing GI lesions could be detrimental. Dr. Feldman in the accompanying article discusses the COX-2 therapeutic hypothesis in more detail.

In summary, great strides have recently been made on the structure and genetic regulation of the two COX isoforms. Significant advances have also been made in defining the role played by each in normal biological processes and disease. However, many unanswered questions still remain regarding the cellular sources of inducible PG synthesis in GI inflammation and cancer and the therapeutic value of recently developed COX-2 selective NSAIDs.

Suggested Reading

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An excellent review covering many aspects of COX structure, inhibition, regulation and role in physiology and disease. ■

Cyclooxygenases and GI Mucosal Protection

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Key Concepts

- *Cyclooxygenases, particularly cyclooxygenase-1 (COX-1), are important in protecting the gastrointestinal mucosa by catalyzing synthesis of mucosa-protective prostaglandins.*

- *Inhibitors of COX-1, such as aspirin and non-steroidal anti-inflammatory drugs (NSAIDs), reduce endogenous PG synthesis and increase the incidence of gastrointestinal ulceration.*

- *Highly selective inhibitors of COX-2 are anti-inflammatory and analgesic, with reduced GI toxicity. However, the safety and cost-effectiveness of these agents is under ongoing evaluation.*

Introduction

Prostaglandin H synthase, more commonly referred to as cyclooxygenase, or COX, is the rate-limiting enzyme for cellular synthesis of prostaglandins (PGs) and thromboxane A_2 (TxA_2). Arachidonic acid, the precursor of endogenous PGs and TxA_2, is a polyunsaturated fatty acid (C20:4) that is a component of phospholipid in cell membranes throughout the body. Under an appropriate stimulus, arachidonic acid is released from the cell membrane by the action of the enzyme phospholipase A_2. Arachidonic acid is then converted to either prostanoids (PGs, TxA_2) via this prostaglandin H synthase (COX) pathway and/or to leukotrienes via an alternate 5-lipoxygenase (5-LOX) pathway.

The enzyme prostaglandin H synthase (COX) actually performs two sequential reactions: a cyclo-oxygenase reaction, which converts arachidonic acid to PGG_2, followed by a peroxidase reaction, which converts PGG_2 to PGH_2. In the GI mucosa, PGH_2 is then converted to various prostaglandins, including PGE_2, $PGF_{2\alpha}$, and PGI_2 (prostacyclin) and, to a lesser extent, to PGD_2. Platelets, on the other hand, convert PGH_2 to thromboxane A_2. Leukocytes convert arachidonic acid to PGs (such as PGE_2) via the COX pathway and to leukotrienes via the 5-LOX pathway. The chemistry of COX is described in more detail by Drs. Mifflin and Powell in the accompanying article.

Acetylsalicylic acid (aspirin) irreversibly blocks COX activity by acetylating a serine residue near the active site of the enzyme. Non-salicylate nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin, naproxen, and ibuprofen reversibly inhibit COX activity by binding at sites different than the aspirin site. Acetylation of the key serine moiety of COX by aspirin prevents arachidonic acid from reaching the active (catalytic) site of COX. Because the platelet is not nucleated, it cannot generate new enzyme after its COX has been irreversibly acetylated and inactivated by aspirin. Thus, thromboxane A_2 production from arachidonic acid is curtailed for the life of the platelet (7 to 10 days). Nucleated cells, such as GI epithelial cells, can produce COX mRNA and new COX protein, permitting gradual restoration of COX-catalyzed PG synthesis once the aspirin has been excreted or metabolized to salicylate. It is

remarkable that aspirin remains in the bloodstream for around three hours after oral dosing, with presumably transient suppression of mucosal PG synthesis, yet once-a-day, low dose aspirin therapy results in significant gastric mucosal damage, as described below.

Cyclooxygenase and GI Mucosal Cytoprotection

PGs such as PGE_2, produced from arachidonic acid in the normal GI tract mucosa by the actions of cyclooxygenase, play a critical role in protecting both the mucosa of stomach and small intestine against injury. The evidence that endogenous GI prostaglandins are "cytoprotective" is two-fold.

First, when GI mucosal synthesis of PGs is blocked by NSAIDs that inhibit COX, ulcers frequently develop in the stomach and/or small intestine of both humans and experimental animals. Furthermore, when humans and experimental animals given NSAIDs are co-treated with PGE analogs, there is remarkable protection against GI ulcers. In animals, low doses of PGE analogs that are cytoprotective against NSAID-induced ulcers do not decrease gastric acid secretion. In humans, the PGE analog most studied, misoprostol, does reduce gastric acid secretion in doses that prevent NSAID ulcers. However, the protective effect of misoprostol on gastric ulcer formation in NSAID users is most likely a consequence of replacement of PGE and not simply a consequence of acid secretion inhibition. This is because histamine-2 receptor antagonists such as ranitidine or famotidine, which are equipotent or even slightly more potent than misoprostol in inhibiting gastric acid secretion in humans, are not as effective as

misoprostol in preventing human gastric ulcers caused by NSAIDs. The observation that NSAIDs also cause ulcers in the jejunum and ileum implies that gastric acid is less important than prostaglandin depletion in the pathogenesis of NSAID ulcers. An exception appears to be NSAID-induced duodenal ulcers in humans which are readily prevented with histamine-2 receptor antagonists or misoprostol, indicating that gastric acid may play a role in the pathogenesis of this type of ulcer. Profound inhibition of gastric acid secretion by proton pump inhibitors in humans virtually eliminates duodenal ulcers caused by NSAIDs and also reduces, but does not eliminate, NSAID-induced gastric ulcers.

A second observation which indicates that endogenous GI PGs are cytoprotective is that lethal GI ulcers develop in experimental animals in whom PGs have been depleted by specific PG antibodies. GI ulcers in rabbits or dogs can be produced by active or passive immunization against PGE_2, $PGF_{2\alpha}$, PGD_2, or 6-keto $PGF_{1\alpha}$. These animal experiments indicate that deficiency of even a single endogenous prostaglandin promotes the development of ulcers in the stomach and intestine.

COX-1 and COX-2

In 1991, it was discovered that there are two different isoforms of COX, COX-1 and COX-2. Differences between COX-1 and COX-2 are described in the accompanying article by Drs. Mifflin and Powell. Briefly, COX-1 is present in virtually all tissues, is expressed at a fairly constant level, and plays a physiological role in several tissues ("housekeeper"). For example, COX-1 in platelets catalyze thromboxane

A_2 production, which leads to platelet aggregation and vasoconstriction, aiding the hemostatic process. Likewise, constitutive expression of COX-1 in the gastrointestinal (GI) tract mucosa is responsible for production of PGs such as PGE_2 which, through a variety of mechanisms (enhanced bicarbonate and mucus secretion, increased mucosal blood flow, increased cell proliferation, and perhaps others), protect the mucosa against ulceration. NSAIDs that interfere with the action of COX-1 reduce constitutive prostanoid synthesis in these tissues and, as a consequence, have the potential to interfere with the normal physiologic processes that prostanoids mediate. Thus, side effects of NSAIDs that inhibit COX-1 include excessive bleeding through impaired platelet-mediated hemostasis, and GI ulcer formation through impaired GI mucosal "cytoprotection." A combination of these two toxicities may result in life-threatening bleeding ulcers. Unfortunately, aspirin and traditional NSAIDs inhibit COX-1 at customary doses and thus may cause life-threatening ulcer complications.

COX-2, unlike COX-1, is present in undectable or very low amounts in most tissues, except kidney and brain. However, COX-2 production can be increased dramatically in cells by inducers of this enzyme, particularly bacterial lipopolysaccharides (endotoxin) and certain cytokines and growth factors. At inflammatory sites, cytokines such as tumor necrosis factor alpha (TNF_α) and interleukin-1 (IL-1) can induce synthesis of large quantities of COX-2, which enhances local synthesis of PGs by inflammatory cells. Thus, NSAIDs that interfere with the activity of induced COX-2 have the potential to
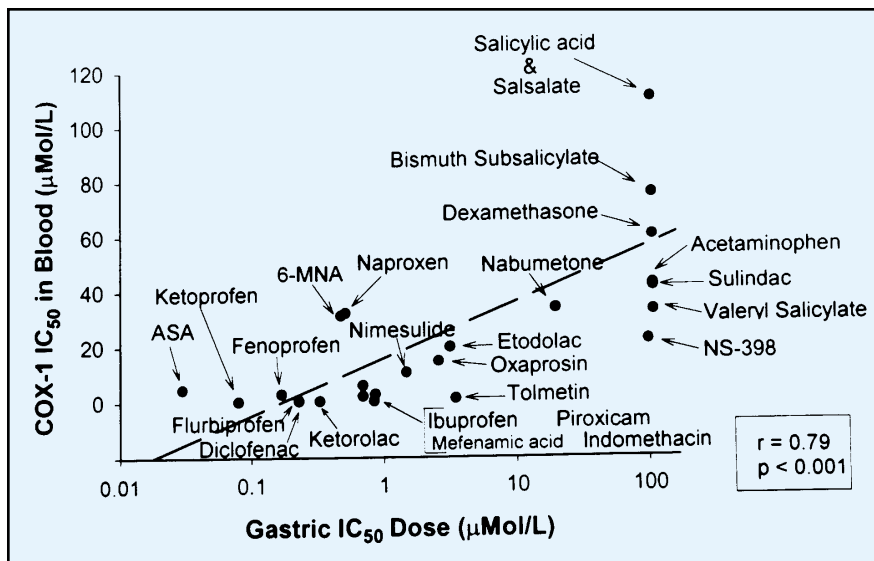
Figure 1. Correlation of gastric IC_{50} with COX-1 IC_{50} in blood for 25 different NSAIDs and anti-inflammatory/analgesic compounds. ASA = acetysalicylic acid (aspirin); 6-MNA = 6-methoxy napthalene acetic acid, the active metabolite of nabumetone (Relafen®). From Cryer and Feldman (see references). Published with permission from *The American Journal of Medicine,* Excerpta Medica, Inc., New York, NY.

reduce local PG production at sites of inflammation, thus ameliorating the inflammatory response. If, however, the same NSAID also blocks COX-1 (i.e., the NSAID is not specific for COX-2 at the dose employed), side effects from COX-1 inhibition discussed earlier are possible. Even highly COX-2 selective NSAIDs such as diclofenac may reduce COX-1 activity at clinically prescribed doses and cause COX-1 related toxicity. What is desired is a COX-2 *specific* agent that has little or no COX-1 effect at clinically prescribed doses. Such an agent could prove to be anti-inflammatory, anti-proliferative, analgesic, and/or antipyretic, yet free of GI toxicity. However, a COX-2 specific drug may have a unique toxicity that is not presently apparent. For example, COX-2 may play a role in renal development, in the renin-angiotensin system, in ovulation, and in uterine function during pregnancy.

COX-2 induction by tissue growth factors (e.g., epidermal growth factor or basic fibroblast growth factor) may be critical in normal wound healing. For example, the expression of COX-2 mRNA is induced at the edges of gastric ulcers in rodents, but not in the adjacent non-ulcerated mucosa. This interesting observation suggests that PGs generated by newly generated COX-2 protein may play an important role in the ulcer healing process, and that interference with this pathway by a selective COX-2 inhibitor may impair healing. Thus, a peptic ulcer caused by the bacterium *Helicobacter pylori* or by a COX-1 inhibitor (such as low-dose aspirin) conceivably could heal less rapidly if a specific COX-2 inhibitor is also being used. COX-2 induced by growth factors may also play a role in tumor growth. Thus, COX-2 inhibitors could be useful as anti-tumor agents.

Acetaminophen (Tylenol, others) is a fairly potent inhibitor of bacterial lipopolysaccharide-induced COX-2 activity in human white blood cells, with an IC_{50} similar to the IC_{50} for aspirin (between 10 and 15 μM). Unlike aspirin, acetaminophen has no inhibitory effect on gastric COX activity. Thus, acetaminophen is technically a selective COX-2 inhibitor with no GI toxicity.

The COX-2 Therapeutic Hypothesis

The current strategy for clinical research in this field has been to (a) develop COX-2 specific or highly selective drugs, (b) test them for efficacy in disease states (e.g., osteoarthritis or rheumatoid arthritis) that have been benefited by conventional, non-selective, COX-inhibiting NSAIDs, and then (c) determine whether the incidence of GI toxicity with the COX-2 selective drug is less than the comparison (non-selective) drug. The COX-2 hypothesis is that the COX-2 selective inhibitors will maintain clinical efficacy without GI toxicity.

Unfortunately, there is no uniform way of defining or determining the COX-2 selectivity of an NSAID. In the past few years, many investigators around the world (including my own laboratory) have utilized fairly standard whole blood assays to address this issue. A venous blood sample is obtained and then exposed to the drug in question as it is allowed to clot. The ability of increasing concentrations of the drug to reduce serum thromboxane B_2 (TxB_2) generation during clotting in a test tube may be used as a COX-1 assay because virtually all of the TxB_2 found in serum during clotting is derived from constitutive COX-1 in platelets. The drug concentration which reduces serum TxB_2 by 50% is referred to as the COX-1 IC_{50}. Our laboratory has recently shown that there is a correlation between the ability of a drug to inhibit COX-1 in whole blood and its ability to reduce gastric mucosal PGE_2 synthesis (Fig. 1). This correlation is not surprising, since the majority of COX in the normal gastric mucosa is the

COX-1 isoform. We have also found that the IC_{50} for gastric COX in human gastric mucosa for NSAIDs such as aspirin corresponds very closely with the IC_{50} *in vivo*. Thus, effects of new COX inhibitors on the stomach can be predicted to some extent by an COX-1 assay of whole blood.

In addition to measuring the whole blood COX-1 IC_{50} for a given NSAID, the ability of increasing concentrations of the same drug to reduce PGE_2 synthesis when a blood sample is exposed to bacterial lipopolysaccharide (endotoxin) can also be determined. Virtually all of the PGE_2 produced in blood under these experimental conditions is derived from COX-2 that has been induced by endotoxin in leukocytes, particularly blood monocytes. Once the COX-2 IC_{50} is determined, the ratio of IC_{50}'s for the drug in question is then calculated (COX-2/COX-1). A ratio close to one indicates little or no COX selectivity. Some examples of nonselective NSAIDs are given in Table 1. An NSAID with a low COX-2/COX-1 ratio is COX-2 selective; some examples are listed in Table 1. Some of the new COX-2 selective inhibitors have COX-2/COX-1 ratios in human whole blood assays of 0.1 or less. Examples include rofecoxib, celecoxib, DuP-697, flosulide, flurbinitroxybutylester, L-745,337, meloxicam, nimesulide, NS-398, and SC-58125. However, there are two reasons to question the idea that COX-2 selective drugs will not be toxic to the GI tract. First, some currently marketed NSAIDs that are at least 10-fold COX-2 selective (COX-2/COX-1 IC_{50} ratio < 0.1) are still able to inhibit COX-1 in the blood and in the stomach at clinically prescribed doses and concentrations (Table 2). Thus, even though nimesulide and diclofenac reduce COX-2 activity at much lower

Table 1. Examples of NSAIDs that have little or no COX-2 Selectivity or are COX-2 Selective in Human Whole Blood Assays

Little or no COX-2 Selectivity		COX-2 Selective	
Naproxen	6-MNA*	Diclofenac	Meloxicam
Indomethacin	Aspirin	Nimesulide	Celecoxib
Ibuprofen	Ketoprofen	NS-398	Rofecoxib

* 6-methoxy naphthalene acetic acid, the active metabolite of nabumetone (Relafen®).

Table 2. Therapeutic Serum Concentrations After Usual Dosing and Human Gastric Mucosal IC_{50} of Two COX-2 Selective NSAIDs Listed in Table 1.

NSAID	Therapeutic Concentration (μM)	Gastric IC50 (μM)	Ratio*
Nimesulide	14.6	1.50	10
Diclofenac	6.1	0.23	27

* Ratio of therapeutic concentration to gastric IC_{50}. From reference by Cryer and Feldman.

concentrations than they inhibit COX-1 activity in whole blood, nimesulide and diclofenac concentrations at customary doses are still well above the IC_{50} for gastric COX. The second reason to question the ultimate GI safety of COX-2 selective inhibitors is that two of these agents, meloxicam (whole blood IC_{50} ratio of 0.09) and nimesulide (whole blood IC_{50} ratio of 0.02–0.06), have already been evaluated in several clinical trials, and the anticipated GI safety has not been realized. Several studies of meloxicam were published in a supplement to *British Journal of Rheumatology* in 1996; interested readers are referred to this supplement for more details. The largest of these studies compared meloxicam to naproxen (a non-selective COX inhibitor) in 370 patients with rheumatoid arthritis who were treated for 6 months. Adverse GI effects occurred in 35.5% of patients on 750 mg/day naproxen and in 26.6% of patients on 7.5 mg/day of meloxicam, an insignificant difference. Several studies of nimesulide were published in the *European*

Journal of Rheumatology and Immunology in 1994. One such study compared nimesulide to naproxen for 2 weeks in 200 patients with tendonitis and bursitis. Adverse GI events occurred in 16% with 200 mg/day nimesulide and 22% of patients on 1100 mg/day of naproxen, an insignificant difference.

Two new COX-2 selective agents have recently been approved in the United States. Celecoxib (Celebrex®) was approved in 1998 for osteoarthritis and rheumatoid arthritis. Rofecoxib (Vioxx®) was approved in 1999 for osteoarthritis and for the short-term management of acute pain in adults, including menstrual pain. Very little data is published as yet on these compounds, but early reports indicate that both drugs (a) cause slightly, but not significantly, more upper GI ulcers detected by endoscopy than placebo; (b) cause much fewer ulcers detected by endoscopy than equally-effective doses of traditional NSAIDs; (c) cause significantly fewer clinically important ulcer events, including GI bleeds,

than traditional NSAID comparators. Serious ulcer events such as bleeding have been reported with both celecoxib and rofecoxib, although cause and effect have not been established. Careful post-marketing surveillance studies will be needed to determine whether celecoxib and rofecoxib prove the COX-2 hypothesis.

Suggested Reading

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Cyclooxygenase-2 and Colon Carcinogenesis

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Key Concepts

- *Colorectal cancer is the second leading cause of cancer death in the U.S.*
- *Regular NSAID use appears to reduce the risk of developing colorectal cancer.*
- *There are data suggesting that NSAIDs reduce the risk of developing colorectal cancer by inhibiting cyclooxygenase-2 (COX-2).*
- *Selective COX-2 inhibitors show promise as chemopreventive agents with fewer gastrointestinal side effects; however, their safety record is not yet proven.*

Introduction

Colorectal cancer is a major cause of illness and death in the United States and other parts of the world. In 1998 there were over 130,000 new cases of colorectal cancer and about 55,000 deaths from the disease (Table 1). Americans have a 1 in 20-lifetime risk of developing colorectal cancer, and approximately one in ten has a family member who develops this disease. Epidemiologic research indicates that there is a 40–50% reduction in mortality from colorectal cancer in persons who take aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs) on a regular basis. Clearly, an effect of this magnitude could have a significant impact on health care, both in terms of lives saved and health care dollars recovered. A

number of groups have initiated research efforts focused on elucidating the molecular basis of the anti-neoplastic effects of aspirin and other NSAIDs. Many of these efforts suggest that inhibition of the enzyme cyclooxygenase-2 (COX-2) by NSAIDs plays some role in cancer risk reduction. Hopefully, continued study of the role of the cyclooxygenase enzymes in colorectal carcinogenesis will determine whether COX-2 selective inhibitors can be used in future cancer prevention regimens.

Evidence for the role of COX in colon cancer prevention

Risk reduction in human sporadic colorectal carcinoma

Of the several observational studies of the effects of exposure to NSAIDs (usually aspirin) and the subsequent development of colorectal cancer, all but one have demonstrated a protective effect of NSAIDs. The studies were performed in a variety of settings in the US and Australia, utilizing both colorectal cancer occurrence and mortality as outcomes. In the studies, exposure to NSAIDs was measured by interview or computerized pharmacy records. In the Nurses Health Study, a protective effect was seen only after 10–15 years of aspirin use. Similar studies have revealed a protective effect of NSAIDs in relation to adenomatous polyp detection. Additionally, a small number of observational studies have shown a significant risk reduction with use of non-aspirin NSAIDs.

The effect of aspirin use on the development of colorectal cancer has been assessed in a randomized clinical trial that had a principal goal of evaluating aspirin for the prevention of myocardial infarc-

tion. A secondary analysis of this study of 22,071 male physicians randomized to placebo or aspirin 325 mg every other day demonstrated no protective effect against the development of colorectal cancer. It is possible that certain characteristics of the study group (such as diet, exercise regimen, age, and gender) or the relatively low dose of aspirin could have obscured a protective effect.

Unfortunately, the low frequency of colorectal cancer makes a large scale randomized clinical trial financially and temporally difficult. More definitive recommendations concerning aspirin use likely will be based on the results of an ongoing randomized clinical trial of aspirin use which utilizes adenomatous polyp incidence as an intermediate endpoint. This multi-center study tests the effect of aspirin at one of two doses versus placebo on the development of adenomatous polyps among patients who have undergone prior colonoscopy with polypectomy. Hopefully, data from that study will help determine the degree of benefit and optimal dose of aspirin in a chemopreventive regimen.

NSAID use and reduction of adenoma size and number in FAP patients

Familial adenomatous polyposis (FAP) is an autosomal dominant inherited disease with variable phenotypic expression that is associated with an increased risk of colorectal cancer at a young age. FAP is responsible for only 1% of colorectal carcinomas detected in the general population. The genetic mutation responsible for this disease resides in the adenomatous polyposis coli (APC) gene. Somatic mutations in the APC gene have been reported also in up to 50% of spontaneous colorectal cancer. Wadell and Loughry first reported

Table 1. U.S. Colorectal cancer statistics (estimated) by gender for 1998.

	Men	Women	Total
Estimated # of new crc cases	64,600	67,000	131,600
% of all new ca cases*	10%	111%	10%
Rank vs other ca types*	3rd (behind lung and prostate)	3rd (behind lung and breast)	2nd overall
Estimated # deaths from crc	27,900	28,600	56,500
% of all ca deaths	9%	11%	10%
Rank vs other causes of ca death	3rd (behind lung and prostate)	3rd (behind lung and breast)	2nd overall

Abbreviations: crc= colorectal cancer; ca= cancer; vs = versus.

* Excluding non-melanoma skin cancers and non-bladder carcinoma-in-situ.

Data from Landis, S.H., et al., Cancer Statistics, 1998. *CA Cancer J. Clin.* 48:6–29.

that regular use of the NSAID sulindac led to regression of rectal adenomas in four patients with FAP, and this phenomenon was confirmed in several other case reports. This observation was then confirmed in randomized, placebo controlled, double-blinded, crossover studies of sulindac use in FAP patients. These studies, collectively, indicate that sulindac has a significant effect on polyp regression in FAP patients.

Potential mechanisms for chemoprevention of intestinal tumors by aspirin and other NSAIDs

Inhibition of cyclooxygenases

The anti-inflammatory properties of NSAIDs are most likely due to their inhibition of cyclooxygenase enzymes. These enzymes catalyze key steps in the conversion of arachidonic acid to endoperoxide (PGH_2), which is a substrate for a variety of prostaglandin synthases which catalyze the formation of prostaglandins and other eicosanoids (see accompanying article by Drs. Mifflin and Powell). Two isoforms of cyclooxygenase have been identified to date, each possessing simi-

lar activities, but differing in expression characteristics and inhibition profiles by NSAIDs. COX-1 mRNA and protein are expressed constitutively in many tissues. A second, inducible isoform of cyclooxygenase, referred to as cyclooxygenase-2 (COX-2) was independently cloned by two groups. COX-2 expression is induced by a number of extracellular and intracellular stimuli. The formation of COX-2 protein parallels the increase in prostaglandin production following stimulation with mitogens or tumor promoters in a wide variety of cell types.

Does dysregulation of COX-2 expression coincide with development of gastrointestinal malignancy? We have previously reported increased COX-2 expression in human colorectal adenocarcinomas when compared to normal adjacent colonic mucosa; these findings have been confirmed by other investigators using different techniques and patient populations. Additionally, COX-2 mRNA and protein levels are increased in intestinal tumors that develop in rodents following carcinogen treatment and in adenomas taken from multiple intestinal neoplasia *(Min)*
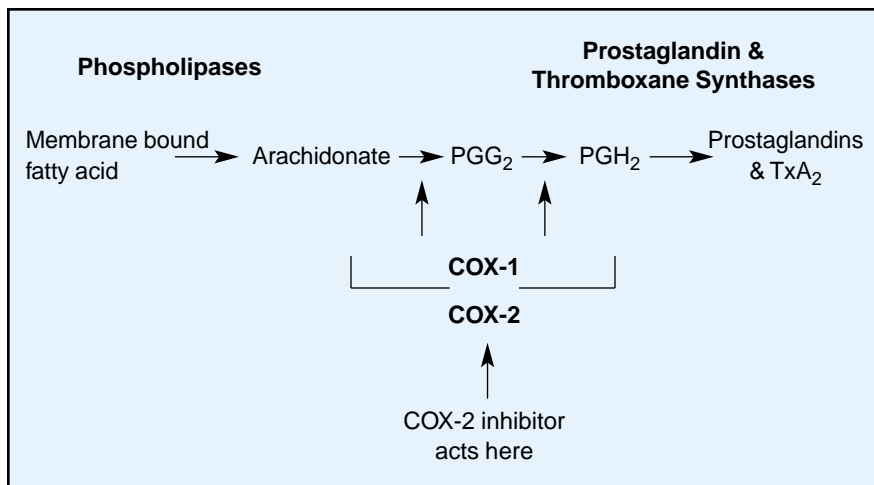
Figure 1. Role of the cyclooxygenase enzymes in prostaglandin synthesis, and putative site of action of selective COX-2 inhibitors.

mice. When intestinal epithelial cells are forced to express COX-2 constitutively, they develop phenotypic changes which include increased adhesion to extracellular membrane (ECM) and a resistance to butyrate-induced apoptosis. Both of these phenotypic changes are consistent with an increased neoplastic potential. COX-2 expression has been detected in 80–90% of colorectal adenocarcinomas, but in only 40-50% of premalignant adenomas. These data suggest that elevation of COX-2 expression is secondary to other initiating events, such as mutations of the APC gene or possibly dysfunction of other genes. (Fig. 1)

Our observation of elevated COX-2 expression in three different models of colorectal carcinogenesis have led us to consider the possibility that COX-2 expression may be related to colorectal tumorigenesis in a causal way. Work by two independent groups has shown a reduction in tumor multiplicity in *Min* mice treated with sulindac or piroxicam, both potent cyclooxygenase inhibitors. Recent studies have demonstrated a significant reduction in premalignant and malignant lesions in carcinogen-treated rats that were given a selective COX-2 inhibitor.

Another study has provided compelling genetic evidence which directly links COX-2 expression to intestinal tumor promotion. This report shows that $APC^{\Delta 716}$ mice, which develop hundreds of tumors per intestine, bred with COX-2 null mice have an 80–90% reduction in tumor multiplicity in the homozygous COX-2 null offspring. These results suggest that: 1) COX-2 may act as a tumor promoter in the intestine; and, 2) increased levels of COX-2 expression may result directly or indirectly from disruption of the APC gene.

Recently, a new class of NSAIDs has been developed. These drugs are highly selective for inhibition of the COX-2 enzyme, but do not strongly inhibit COX-1. They were primarily developed as anti-inflammatory agents with the goal of lessening the gastrointestinal side effects caused by inhibition of COX-1. These selective COX-2 inhibitors have proven effective in inhibiting tumor growth in animal studies, and these agents also have been shown to possess anti-angiogenic activity *in vitro* which may contribute to their anti-neoplastic effects *in vivo.* Although the safety profiles of these drugs are not clearly established, they may prove to be effective chemopreventive agents with fewer gastrointestinal side effects than non-selective NSAIDs.

Cyclooxygenase independent mechanisms

Epidemiologic data strongly support the chemoprotective effects of NSAIDs on gastrointestinal malignancies, while the data supporting their benefit in other solid tumors are not as well developed. The precise mechanism by which NSAIDs prevent and/or cause regression of colorectal tumors is not known. Despite different chemical structures, inhibition profiles, and drug half lives, all NSAIDs in clinical use possess cyclooxygenase inhibitory activity. Some investigators have reported effects of NSAIDs which likely are not due to their inhibition of cyclooxygenase activity. 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). By using cyclooxygenase-deficient cell lines or drug metabolites lacking COX-inhibitory activity, these studies rule out the involvement of cyclooxygenase enzymes in the growth inhibitory effect. Certainly, this class of drugs appears to affect biochemical pathways unrelated to cyclooxygenase enzymes, and these effects are likely to occur in a dose-dependent fashion (some effects occurring only at toxic doses). The specific mechanisms of these COX-independent effects, and their therapeutic implications, are not yet well understood.

Risks of chronic NSAID therapy for cancer prevention

The role of NSAIDs for gastrointestinal cancer prevention will be determined by the side effects of chronic use of these

drugs. There are concerns about the safety of long term use of aspirin and other NSAIDs in humans. Long term aspirin use can result in serious gastrointestinal and renal adverse effects, even at relatively low doses of drug. These side effects tend to increase in older patients. The most significant side effects, in terms of morbidity and mortality, are gastrointestinal, and include dyspepsia, peptic ulcer, and gastrointestinal bleeding. It is estimated that regular users of NSAIDs have roughly a three-fold greater relative risk of developing serious gastrointestinal complications when compared to non-users of NSAIDs. Furthermore, the consequences of NSAID-associated gastrointestinal complications are often dire. A British study of 235 patients with severe peptic ulceration found that the mortality rate from ulceration among regular users of NSAIDs was greater than twice that of non-users.

The cost of NSAID-associated complications also has been considerable. A retrospective cohort study of 75,350 elderly (>65) Tennessee Medicaid recipients by Smalley, et al., found that the adjusted mean annual payment for medical care related to gastrointestinal disorders was $134 for non-users of NSAIDs and was $244 among regular users of NSAIDs. These findings may well underestimate the actual, current costs of NSAID-related gastroenteropathy, as omeprazole and misoprostol were not on the Medicaid formulary at that time.

As new data become available, we must constantly reassess the risks versus the benefits of chronic NSAID therapy. The aforementioned side effects of aspirin therapy may preclude prophylactic use of that drug in all but the highest risk populations. The deleterious effects of non-selective NSAIDs on the gastrointestinal tract, including gastric erosions, ulcerations, and blood loss, are postulated to result from inhibition of COX-1. While COX-2 selective NSAIDs are available, these drugs do not yet have established safety profiles. The side effects of any chemoprotective agent must be low to insure compliance and to achieve the desired result, since the absolute risk of colorectal cancer in the general population is quite low. On the other hand, if high risk populations can be identified readily, then the use of these agents in those populations may be more reasonable, because of a much more favorable risk to benefit ratio.

The risk-benefit ratio for chemoprevention of colorectal cancer likely would improve if accessible techniques were available to identify groups that are at high risk for the subsequent development of colorectal cancer. Advances in the discovery and testing of colorectal cancer genes hopefully will make the identification of cohorts at high risk for developing cancer more likely. Additionally, if agents such as the selective COX-2 inhibitors prove to have fewer adverse effects than non-selective NSAIDs, the risk to benefit ratio might improve. Human clinical trials evaluating the anti-neoplastic effects of selective COX-2 inhibitors will need to be completed before the role of these agents in cancer prevention can be determined.

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The Regulatory Peptide Letter

Prepared with the assistance of a grant from Novartis, *The Regulatory Peptide Letter* bridges the gap between basic research and clinical application.

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