Prostaglandin endoperoxide synthase: Why two isoforms?

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Williams, Christopher S., and Raymond N. DuBois. Prostaglandin endoperoxide synthase: Why two isoforms? Am. J. Physiol. 270 (Gastrointest. Liver Physiol. 33): G393-G400, 1996.-Prostaglandin endoperoxide synthase-1 [prostaglandin G/H synthase-1 (PGHS-1)] and PGHS-2 are key enzymes in the conversion of arachidonic acid to prostaglandins and other eicosanoids. We refer to these isoforms as cyclooxygenase-1 (COX-1) and COX-2 in this review. This brief review focuses on recent developments in the study of these enzymes. Alterations in the expression levels of COX-2 result in distinct phenotypic changes in intestinal epithelial cells. Overexpression of COX-2 in intestinal epithelial cells results in increased adhesion to extracellular matrix proteins and inhibition of apoptosis. Disruption of the COX-2 gene in mice results in renal dysplasia, cardiac fibrosis, and defects in the ovary. Interestingly, disruption of the COX-1 gene results in distinct phenotypic changes different from those observed for COX-2. COX-1 null mice survive well, have no gastric pathology, and show less indomethacin induced gastric ulceration than wild-type mice. These two closely related enzymes must have distinct functions in the organism, since lack of their expression causes distinct phenotypic changes for each respective isoform.

cyclooxygenase: intestinal epithelial cells; eicosanoid; colorectal cancer

ARACHIDONIC ACID PRODUCTS such as prostaglandins, thromboxanes, and 15-hydroxyeicosatetraenoic acids (HETEs) are collectively referred to as eicosanoids. These products are known to be involved in such processes as inflammation, ovulation, modulation of immune responses, and mitogenesis. To understand the role of arachidonic acid metabolites in these processes it is important to understand which downstream signaling pathways are affected. The regulation of eicosanoid production has become an area of intense study because these fatty acid metabolites affect multiple signaling pathways that modulate a wide range of physiological functions.

There are multiple steps in the biosynthetic pathway for eicosanoids that are possible sites of regulation (Fig. 1). The first step is the liberation of arachidonic acid from membrane phospholipid by phospholipase. Several phospholipases have been characterized, including cardiac phospholipase Δ2 (PLA2) and secretory PLA2. Of interest, one of the phospholipase genes was recently shown to act as a modifier (Mom1) in multiple intestinal neoplasia (Min) mice. Min mice have a mutated gene [adenomatous polyposis coli (APC)], which leads to the development of multiple intestinal tumors (67). The gene for secretory PLA2 was recently reported as one of the modifier genes that alters the number of intestinal tumors when affected (39). Phospholipases were thought to be the most important regulatory step in prostaglandin production.

Following liberation from phospholipid, free arachidonic acid is converted to prostaglandin C/H2 (PGE2), the upstream metabolite for prostaglandins and thromboxanes via prostaglandin G/H synthase-1 (PGHS-1) (8,11,14-eicosatrienoate, hydrogen donor: oxygen oxidoreductase, EC 1.14.99.1), which is referred to as cyclooxygenase-1 (COX-1) in this review (Fig. 1). This enzyme was purified from bovine vesicular glands by Miyamoto et al. (43) in 1976. COX-1 mRNA and protein are present at relatively stable levels in many tissues, but prostanoid production in these tissues is variable. Therefore, it was originally thought that conversion of arachidonate to PGE2 was not a likely regulatory step, and that regulation occurred either at arachidonate release or at some distal step in the metabolic pathway. In 1989 Simmons et al. (62) identified a second, inducible form of cyclooxygenase, known as prostaglandin endoperoxide synthase-2 (COX-2). This cyclooxygenase isoform was independently identified by differential screening of a phorbol ester-stimulated Swiss-3T3 fibroblast cDNA library (33) and subsequently by many other groups. COX-2 expression is inducible by a wide range of extracellular and intracellular stimuli, including lipopolysaccharide (15, 37, 51), forskolin (34), interleukin-1 (IL-1), tumor necrosis factor (TNF) (7, 16, 27),...

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Fig. 1. Arachidonate metabolism via cyclooxygenase pathway. Schematic representation of the regulation of arachidonic acid metabolism as it relates to cyclooxygenase enzymes. AII, angiotensin II; LPS, lipopolysaccharide, TGF-α and TGF-β, transforming growth factor-α and -β, respectively; IL-1β, interleukin-1β; TPA, 12-O-tetradecanoylphorbol 13-acetate; PDGF, platelet-derived growth factor; Dexa, dexamethasone; TNF-α, tumor necrosis factor-α; PMA, phorbol 12-myristate 13-acetate; CHX, cycloheximide; FSH, follicle stimulating hormone; LH, luteinizing hormone; RSV, Rous sarcoma virus; PAF, platelet-activating factor; AA, arachidonic acid; EGF, epidermal growth factor; IL-2, interleukin-2; VSMC, vascular smooth muscle cells.

serum (8, 58), epidermal growth factor (EGF) (22), synaptic activity (74), transforming growth factor-α (TGF-α) (11), human chorionic gonadotropin (63), interferon-γ (56), platelet activating factor, retinoic acid (3), and endothelin (29). Additionally, the formation of COX-2 protein parallels the increase in prostaglandin production that is commonly the result of mitogenic stimulation in a wide variety of cell types. Prostaglandin production increases rapidly in rat intestinal epithelial cells (RIE) after stimulation with mitogens such as TGF-α or EGF (11). Under resting conditions, these cells have undetectable COX-2 levels; hence prostaglandins are not being produced by this enzyme. The regulation of COX-2 expression is a key step in prostaglandin production in rat intestinal epithelial cells.

If one assumes that these two enzymes have similar or identical activities, then one obvious question to ask is, Why are there two isoforms? Why is one isoform constitutively expressed in most cell types, and the other induced? Though COX-1 and COX-2 produce similar products, it seems likely that there is some reason for the existence of these two distinct, but related, enzymes. One possibility is that these two isoforms provide different roles because they are located in separate subcellular compartments (45). Determining the biological role each isoform plays in the organism is an important question that is currently under intense study.

STRUCTURE

The genomic structure of both human (75) and murine (32) COX-1 is composed of 11 exons and 10 introns spanning 22.5 kb. The human COX-1 locus maps to chromosome 9q32-33.3. By comparison, the human COX-2 gene is only 8.3 kb in size. This reduction in size is primarily due to smaller introns. Most of the exons are conserved, with the exception of the absence of exon 2 in COX-2 (Fig. 2). Fluorescence in situ
hybridization localized human COX-2 to chromosome 1q25.2-q25.3 (31). It is of interest to note that one of the phospholipase genes (PLA2G4) is in extremely close proximity to the COX-2 gene on chromosome 1, located at 1q24-q25. Because secretory PLA2 has been reported as one of the modifier genes of the Min phenotype, the close proximity of COX-2 and PLA2 could have some biological relevance (39).

Homology comparisons of the 5'-flanking regions of the two cyclooxygenase genes reveals little similarity. However, the COX-2 promoter region is partially conserved between species (63%). The human and chicken COX-2 promoters contain a canonical TATA box, whereas the murine and rat promoters do not possess such a sequence. Computerized motif searches suggest that the following transcription factor consensus sequences are present in the COX-2 promoter: CArG box, NF-IL-6 (nuclear factor of IL-6), murine nuclear protein 1 (PEA-1), myb, GATA-1, xenobiotic response element, interferon-λ (PEA-3), and substance P. Interestingly, these sequences are conserved between murine and human COX-2 genes, suggesting an important role for their presence.

After mitogenic stimulation, the level of COX-2 mRNA rapidly increases within 30 min in a cycloheximide-independent fashion (8, 27) and remains elevated for 6–8 h before rapidly declining to baseline levels within 24 h (11). Additional stimulation results in no further COX-2 production, providing a classic example of immediate early or primary response gene activation (46).

The transcription of COX-1 and COX-2 yields mRNAs that are 2.7 and 4.5 kb in size, respectively (Fig. 3). There have been reports of splice variants being produced from the COX-1 primary transcript as a result of TGF-β1, IL-1β, TNF-α, phorbol esters, and serum stimulation (9). There is also some evidence for alternative splicing of the COX-2 mRNA. Xie et al. (73) used chicken embryo fibroblasts that were permanently transfected with temperature-sensitive v-src, a potent COX-2 inducer, to investigate splice variation of the COX-2 mRNA. They observed that in nonproliferating chicken embryo fibroblasts expressing v-src, the predominant COX-2 mRNA possessed an unspliced intron that prohibited translation (73). Upon transformation and mitogenic stimulation, the majority of the COX-2 mRNA produced was of the completely spliced derivative and was translated into COX-2 protein. To our knowledge, additional work characterizing the tissue prevalence of this alternative transcript or the machinery involved in the differential splicing has not been reported.

COX-1 mRNA encodes a 565-residue, 65-kDa protein containing a short signal peptide and four possible N-linked glycosylation sites (Fig. 3). In Fig. 3 the COX-1 and COX-2 cDNA and protein structures are compared in a linear fashion. COX-2, a roughly 70-kDa protein, is ~75% homologous to the COX-1 protein. The cyclooxygenase and peroxidase regions are conserved between the two proteins; however, there are some other differences in the primary amino acid sequence.
The amino terminus is slightly truncated in COX-2 compared with COX-1, and the carboxy terminus of COX-2 possesses an 18-residue insert that is absent from COX-1. It is possible these subtle differences in the primary amino acid sequence may cause differences in the secondary and tertiary structure of the protein that could affect the active site of these two enzymes. For example, COX-1 is completely inhibited by aspirin treatment, whereas COX-2 converts arachidonic acid to 15-R-HETE after aspirin treatment, functioning as a 15-lipoxygenase (4, 36).

**LOCALIZATION**

It is possible that some of the differences in the NH$_2$ terminal or COOH terminal of these isoforms may affect their subcellular localization. Early work using immunohistochemistry revealed no apparent difference in subcellular localization of COX-1 and COX-2 (both cytoplasmic and perinuclear) distribution (54, 55). However, later work using more refined technologies (quantitative confocal fluorescence imaging microscopy and a histofluorescence staining technique capable of localizing cyclooxygenase/peroxidase activity) revealed that COX-1 functioned primarily in the endoplasmic reticulum, whereas COX-2 activity was located in both the endoplasmic reticulum and the perinuclear envelope (45). If COX-1 and COX-2 are located in separate subcellular compartments, then the distribution of their metabolites may also be partitioned. Eicosanoid products produced via COX-2 could be preferentially distributed to the nuclear compartment of the cell and therefore may modulate transcription of target genes. The compartmentalization and differential regulation characteristics may explain the need for two isoforms of the cyclooxygenase enzyme.

**INFLAMMATION**

Nonsteroidal antiinflammatory drugs (NSAIDs) typically are prescribed to control joint discomfort, common aches, and inflammation. NSAIDs decrease prostaglandin production by inhibiting cyclooxygenase activity. Unfortunately, one side effect of prolonged NSAID use is gastric irritation (2, 13, 65). COX-1 mRNA, protein, and activity levels do not change during the inflammatory process. However, COX-2 levels increase dramatically, and it is likely that the increase in eicosanoid production is a result of increased COX-2 activity. Therefore, a drug that selectively inhibits COX-2 activity without affecting constitutive COX-1 activity might be efficacious in controlling inflammation without the side effects commonly seen following complete blockade of prostaglandin production.

Selective COX-2 inhibitors have recently been developed. To test the above hypothesis, one group has employed the rat carrageenan subcutaneous air pouch model (40). This model of inflammation is produced by injecting carrageenan directly into a previously formed subcutaneous air pouch on the back of a rat. Typically, inflammation, as measured by prostaglandin production and volume of exudate produced, occurs within several days postinjection. If selective COX-2 inhibitors (in this case NS-398) are administered, prostaglandin production and subsequent inflammation are significantly reduced, without a concomitant decrease in gastric prostaglandin production or the development of gastric lesions (40). Therefore, preferentially inhibiting COX-2 may have a distinct application in clinical situations in which inflammation is a major component of the disease process and avoidance of gastric complications that develop after NSAID therapy is desired.

**INTESTINAL NEOPLASIA**

Tumorigenesis is a complex process that results from multiple genetic alterations involving the dysregulation of signal-transduction pathways, cell cycle arrest mechanisms, and/or differentiation state determinants. Malignancies do not develop as an a priori genetic anomaly (14). For example, in some hypotheti cal models of spontaneous colorectal cancer, an initial somatic mutation in the APC gene does not transform intestinal epithelial cells (42); however, a subsequent mutation in p53, a potent tumor suppressor, would result in a malignant phenotype.

Preliminary data indirectly suggest that cyclooxygenase activity may have an effect on tumor formation in the intestine. Several lines of evidence converge to support this hypothesis. Aspirin irreversibly inactivates COX-1 through acetylation of serine-530 (61) and alters COX-2 activity by acetylation of serine-516 (36). Several studies revealed a decrease in the relative risk of colorectal cancer that correlates with the continuous use of NSAIDs (19, 20, 38, 68, 70). Recently, a study that focused on prevention of colorectal cancer in women found that long-term aspirin use substantially reduces the risk of colorectal cancer (18). Familial adenomatous polyposis (FAP) is a hereditary disease transmitted in an autosomal dominant fashion, which results in numerous intestinal adenomas during adolescence. These adenomas predictably degenerate into colorectal cancer if the colon is not surgically resected before the second or third decade of life. Genetic linkage studies first identified a locus on chromosome 5q21 as the site of the genetic defect in patients with FAP (30, 48). The APC gene was subsequently mapped to this site, inactivation of which results in FAP. Over 200 germline mutations of the very large (>100 kb) APC gene have been reported, most of which are predicted to result in a truncated protein that is dysfunctional in its role as a tumor suppressor. The precise function of the APC gene is unknown, but the protein encoded by this gene is thought to have important interactions with β-catenin and E-cadherin (1, 25, 26, 57). Sulindac, a commonly prescribed NSAID, reduces the frequency and size of intestinal adenomatous polyps in patients with FAP (17, 49, 66, 72). NSAIDs have other effects not targeted at cyclooxygenases, therefore the observation that NSAID administration causes regression of colonic adenomas possibly involves regulatory pathways distinct from cyclooxygenase. However, other studies have shown an increased level of PGE$_2$ (52, 53), lipid

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peroxidation products (23), arachidonate, and docosa-
hexaenoic acid (47) in colorectal tumors. These findings
further substantiate a possible causal relationship
between cyclooxygenase activity and tumor formation.

Several groups have investigated this possibility
further. We have sought to determine whether cyclooxy-
genase mRNA was upregulated in colon carcinomas
obtained through endoscopic biopsy and surgical resec-
tion. Our laboratory (12) reported that of 14 human
colon carcinomas screened, 12 had increased COX-2
mRNA, and of 14 unpaired adenomas screened, 43% had increased levels of COX-2 mRNA. The decrease in
incidence of elevated COX-2 expression in benign adeno-
amas is not surprising, considering that adenomas are
precursors to carcinomas and are expected to express a
different array of gene products. Indeed, the fact that
there is a marked elevation of COX-2 mRNA in the
adenomas compared with normal adjacent mucosa may
indicate that increased expression of COX-2 is involved
in the progression to a transformed state. Therefore,
the increased COX-2 levels in adenomas may be useful
as a prognostic marker for tumorigenesis in the intesti-
nal epithelium. Studies from other laboratories have
confirmed our findings and shown that COX-2 protein
levels are increased in a significant number of colorec-
tal carcinomas as well (28). However, COX-1 expression
was low in both normal mucosa and tumor samples.
More extensive patient studies will have to be per-
formed before any definite conclusions can be drawn
about the feasibility of utilizing COX-2 levels as a
prognostic marker.

MODELING INTESTINAL TUMORIGENESIS

Colonic epithelial cells in vivo express low levels of
COX-1 and almost undetectable levels of COX-2,
whereas colorectal cancers express 2- to 50-fold higher
levels of COX-2. Immunostaining of colorectal cancer
tissue sections reveals that the bulk of COX-2 protein is
expressed in malignant colonic epithelial cells (59). We
sought to set up an in vitro model of this cyclooxygenase
expression pattern found in colorectal cancer by engi-
near rat intestinal epithelial (RIE) cells to overex-
presses COX-2 (RIE-sense) constitutively (71). We used
normal RIE cells as controls, in addition to a cell line
overexpressing the COX-2 cDNA inserted in the anti-
sense orientation. Appropriate experiments were con-
ducted to confirm that COX-2 was being expressed and
was functional in the RIE-sense cell lines, and absent
from the antisense RIE cells. Intestinal epithelial cells
usually undergo apoptosis in culture after sodium
butyrate treatment (6, 21, 24). RIE cells that are
genetically engineered to overexpress COX-2 have two
interesting phenotypic changes: 1) they do not undergo
apoptosis in response to sodium butyrate treatment as
the normal cells do, and 2) they have increased adhe-
sion to extracellular matrix proteins compared with
normal or antisense cells. These two properties cer-
tainly may be considered important in tumorigenesis
(71). Additional experiments are underway to deter-
mine the molecular basis for these phenotypic changes
involving increased adhesion to extracellular matrix
and inhibition of apoptosis.

COX-1 AND COX-2 GENE DISRUPTION EXPERIMENTS

Gene disruption techniques have recently been util-
ized to understand the biological relevance of a variety
of regulatory molecules including enzymes, cytokines,
growth factors, transcription factors, and cell surface
receptors. Oftentimes abolishing the expression of a
key regulatory gene will result in an embryonic lethal
phenotype. When the lethal phenotype can be avoided,
the resultant phenotypic changes observed in the organ-
ism can help to explain the functional role of a particu-
lar gene. This approach is complicated by the fact that
different phenotypes have been observed for disrupting
identical genes in alternate genetic backgrounds. For
example, no alteration in intestinal function was ob-
served when the EGF receptor was knocked out in one
strain of mice (69). However, severe necrotizing enterocolitis occurred when Swiss Webster Black mice were
utilized for disruption of the EGF receptor (41).

Targeted gene disruption has been employed to gen-
erate COX-1-and COX-2-deficient mice (10, 35). COX-
1(-/-) mice survive well, have no gastric pathology,
and show less indomethacin-induced gastric ulceration
than COX-1 wild-type mice (35). COX-1 null mice also
have reduced platelet aggregation and decreased inflam-
matory response to arachidonic acid, but not to phorbol
ester stimulation. COX-1(-/-) females mated to COX-
1(-/-) males produce few live offspring, which is
probably due to parturition difficulties in the COX-
1(-/-) female. Several distinct phenotypic changes
were observed in the COX-2 null mice: 1) absence of
corpora lutea in the developing ovary by one group (10),
2) renal nephropathy (10, 44), 3) cardiac fibrosis by one
group (10), and 4) increased susceptibility to peritonitis
by another group (44). Clearly, these results implicate
the importance of COX-2 activity in the development of
diverse organ systems.

SUMMARY

The purpose of this brief review is to stimulate more
consideration of the biological role of these enzymes
and to provide an update on the most recent studies
regarding their characterization. COX-1 and COX-2
are key enzymes in the production of a variety of
eicosanoid products. These eicosanoids mediate numer-
ous cellular responses, including modulation of cellular
adhesion, differentiation, and mitogenesis. Continuous
use of NSAIDs in humans leads to a 50% decrease in
the relative rate of colorectal cancer. COX-2 expression
is increased dramatically in a significant number of
colorectal cancers. Overexpressing the COX-2 gene
in intestinal epithelial cells results in increased adhesion
to extracellular matrix proteins and inhibition of apop-
tosis. These phenotypic changes may represent an
increased tumorigenic potential of epithelial cells as a
result of COX-2 upregulation.

Targeted gene disruption approaches have been em-
ployed to help understand the role of numerous genes.
COX-1 and COX-2 null mice have been generated. COX-1 null mice survive well, have no gastric pathology, and show less indomethacin-induced gastric ulceration than wild-type mice. COX-2 null mice exhibit renal abnormalities, cardiac fibrosis, and corpora lutea defects. Disruption of both COX-1 and COX-2 genes has revealed distinct phenotypic changes for each gene, indicating that these isoforms must carry out different functions in the organism. Future work further characterizing these mouse models and developing transgenic animals that overexpress the COX 2 gene in specific segments of the intestine may add to our understanding of these two important enzymes in normal physiology and in tumorigenesis.

We thank Gordon Williams and Ray Dunn for helpful suggestions. Our gratitude goes to Jason Morrow and Walter Smallley for editorial assistance.

This study was supported by the A. B. Hancock, Jr., Memorial Laboratory, the Luisele P. Markby Charitable Trust, National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-47297-02 (R. N. DuBois), and a Department of Veterans Affairs Merit Grant (R. N. DuBois). R. N. DuBois is a recipient of a Veterans Affairs Research Associate career development award and the Boehringer Ingelheim New Investigator Award and is an American Gastroenterology Association Industry Research Scholar.

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REFERENCES


Sirois, J. and J. S. Richards. Transcriptional regulation of the rat prostaglandin endoperoxide synthase 2 gene in granulosa...