Cyclooxygenase-2 Biology

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Abstract: In mammalian cells, eicosanoid biosynthesis is usually initiated by the activation of phospholipase A2 and the release of arachidonic acid from membrane phospholipids in response to the interaction of a stimulus with a receptor on the cell surface. Arachidonic acid is subsequently transformed by the enzyme cyclooxygenase (COX) to prostaglandins (PGs) and thromboxane (TX). The COX pathway is of particular clinical relevance because it is the major target for non-steroidal anti-inflammatory drugs, which are commonly used for relieving inflammation, pain and fever. In 1991, it was disclosed that COX exists in two distinct isozymes (COX-1 and COX-2), one of which, COX-2, is primarily responsible for inflammation but apparently not for gastrointestinal integrity or platelet aggregation. For this reason, in recent years, novel compounds that are selective for this isozyme, the so-called selective COX-2 inhibitors or COXIBs, which retain anti-inflammatory activity but minimize the risk of gastrointestinal toxicity and bleeding, have been developed. This review article provides an overview and an update on the progress achieved in the area of COX-2 and PG biosynthesis and describes the role of COX-2 in health and disease. It also discusses some unresolved issues related to the use of selective COX-2 inhibitors as a safe and promising therapeutic option not only for the treatment of inflammatory states but also for cancer.

Key Words: Eicosanoids, prostaglandins, cyclooxygenase-2, non-steroidal anti-inflammatory drugs, cyclooxygenase-2 inhibitors, gastrointestinal tract.

PROSTAGLANDINS

The history of prostaglandins (PGs) date back in the early 1930’s when Goldblatt and Von Euler who independently discovered a fatty acid in human seminal fluid with potent vasoactive properties in rabbits and guinea pigs [1, 2]. von Euler named this compound prostaglandin because he assumed it was originated in the prostate gland (it is now known to be originated in the seminal vesicles) and suggested that prostaglandins played a role in sperm transport [2]. Thirty years later, Bergstrom and Samuelsson established the structure of the first two PGs which were termed PGE and PGF because of their respective partitions into ether and phosphate buffer (josfat in Swedish) and demonstrated that they were produced from the essential fatty acid arachidonic acid [3]. Today the nomenclature of PGs describes ten specific molecular groups, designated by the letters A through J, that are unique by their variation in the functional groups attached to positions 9 and 11 of the cyclopentane ring [4]. Each group of PGs consists of three series designated by the subscript numbers 1, 2 and 3, depending on the substrate originated: di-homo-γ-linoleic acid, arachidonic acid, and eicosapentaenoic acid, respectively. Among the PGs, the most widely distributed and best characterized are those derived from arachidonic acid (i.e., PGE2, PGD2, PGI2, and PGF2α). For PGF, the additional subscript α or β denotes the spatial configuration of the carbon 9 hydroxyl group. Special mention should be given to the platelet pro-aggregatory and vasoconstrictor molecule, thromboxane (TX) A2, whose structure and biosynthesis was elucidated soon after the discovery of PGs [4].

PROSTAGLANDIN BIOSYNTHESIS

In 1971, Vane [5], Ferreira et al. [6] and Smith et al. [7] published their seminal observations proposing that the ability of aspirin-like drugs to suppress inflammation rests primarily on their ability to inhibit the production of PGs. This work fostered further research with focus on the elucidation of the pathway leading to the biosynthesis of PGs in animal cells, which concluded that the synthesis of PGs from arachidonic acid is initiated by the enzyme cyclooxygenase (COX). Although in human cells there are at least two different COX isozymes designated COX-1 and COX-2 (as discussed below a third COX isoform termed COX-3 has recently been identified), the basic catalytic activities of these isoforms are similar enough to treat them as if they were biochemically identical. Accordingly, the biochemistry of these COX isoforms is herewith discussed as one. Moreover, although other names for COX are PG endoperoxide synthase, PG G/H synthase and PGH synthase, in this review article this enzyme is referred to as COX.

COX is a membrane-bound bifunctional enzyme that catalyzes the first two committed steps in the pathway leading to the formation of PGs and TX, namely cyclooxygenation and peroxidation. In the first step, COX cyclizes and adds two molecules of O2 to arachidonic acid to form the cyclic hydroperoxide PGG2. Subsequently, COX reduces PGG2 to PGH2 [8, 9]. PGH2 is a highly unstable endoperoxide, which functions as an intermediate substrate...
for the biosynthesis, by specific synthases and isomerases, of PGs of the E₃, F₂ and D₃ series and also of PGl₂ (prostacyclin) and TXA₂ [4]. Interestingly, the coupling of PGH₂ synthesis to its transformation to PGs and TX by downstream enzymes is intricately orchestrated in a cell-specific fashion. That is, any given prostanooid-forming cell tends to form only one of these compounds as its major product. Thus, for example, in brain and mast cells, PGH₂ is converted to PGD₂ by cytosolic enzyme PGD synthase. PGH₂ can alternatively be converted to PGF₂α by PGF synthase, which is mainly expressed in the uterus. Vascular endothelial cells produce PGI₂ or prostacyclin from PGH₂ by means of the PGI or prostacyclin synthase, and platelets release TXA₂ from the same precursor (PGH₂) as the PGs through the action of the enzyme TX synthase. Both PGl₂ and TXA₂ have a very short half-life (30 seconds and 3 minutes, respectively) and are rapidly hydrolyzed to the inactive compounds TXB₂ and 6-keto-PGF₁α, respectively. Finally, PGE₁ is formed in many cell types by the enzyme PGE synthase. A schematic representation of these pathways is shown in Fig. (1). It is important to note that PGE synthase is also present in an inducible form (mPGE synthase), which is membrane-bound, glutathione-dependent and whose expression is induced by proinflammatory compounds, such as interleukin-1β [10, 11]. The coordinate induction of multiple enzymes of the prostanooid pathway, such as COX-2 and mPGE synthase, during the inflammatory response is a concept that is currently evolving.

Both cyclooxygenase and peroxidase activities of COX are associated with a single protein molecule and use a single heme moiety within the enzyme [9]. However, the active sites for the cyclooxygenase and peroxidase activities of COX have been shown to be distinct and independent in pharmacological and in vitro mutagenesis studies [9]. Hence, the two active sites for cyclooxygenase and peroxidase probably overlap [9]. The cyclooxygenase active site is proposed to be a long hydrophobic channel, which extends from the membrane binding domain to near the heme group. This hydrophobic channel has been shown to bind arachidonate and other fatty acids as substrates. Thus, in addition to the 20-carbon arachidonate (20:4 n6), COX also uses di-homo-γ-linolenate (20:3 n6), 5,8,11,14,17-eicosapentaenoic acid (EPA), γ-linolenic acid, α-linolenic acid and linolenic acid. EPA is subsequently converted to PGH₂ whereas the 18-carbon fatty acids are converted into mono-hydroxy acids. Although COX-1 and COX-2 oxygenate arachidonate with almost identical kinetics, in general, COX-2 is much more efficient with alternative substrates. For instance, COX-2 utilizes both fatty acids and endocannabinoids, including 2-arachidonylglycerol and anandamide, as substrates. COX-2 action on 2-arachidonoylglycerol and anandamide generates endocannabinoid-derived prostanooids, among them PG glycerol esters and ethanolamides [12]. In addition, aspirin-acetylated COX-2 converts arachidonic acid to 15R-hydroxyeicosatetraenoic acid (15R-HETE), which is further transformed into 15-epi-lipoxins [13]. Moreover, when exposed to aspirin, COX-2-expressing cells enzymatically transform omega-3 docosahexaenoic acid (DHA) to previously unrecognized compounds, i.e., a novel 17R series of hydroxy-DHAs [14]. Both 15-epi-lipoxins and hydroxy-DHAs bear potent anti-inflammatory properties and play a key role in the resolution of inflammation.

COX is an integral membrane protein found mainly in microsomal membranes. Though confocal fluorescence imaging microscopy and histo fluorescence staining techniques reveal that COX-1 and COX-2 are located in the endoplasmic reticulum and nuclear envelope, COX-2 is more highly concentrated in the nuclear envelope [15]. It is unclear why COX is associated with several different membrane systems but one possibility may be that PG synthesis at different subcellular sites is elicited by different stimuli. In any case, the aspirin acetylation site, the site of trypsin cleavage and the major antigenic determinants of COX are on the cytoplasmic side of the endoplasmic reticulum indicating that PGH₂ is generated intracellularly rather than extracellularly. Most of the synthases, which further metabolize PGH₂ also appear to be localized on the endoplasmic reticulum.

**PROSTAGLANDIN RECEPTORS**

Similar to what occurs with the PGH₂ downstream metabolizing enzymes (i.e., PG synthases and isomerases), prostanooid receptors are also cell and/or tissue specific. There are at least 9 known PG receptors as well as several of their splice variants that belong to a subfamily of the G protein-coupled receptor (GPCR) superfamily of seven transmembrane spanning proteins [16]. Four of the receptor subtypes bind PGE₂ (EP₁, EP₂, EP₃), two bind PGD₂ (DP₁ and DP₂) and the rest are single receptors for PGF₂α, PGI₂ and TXA₂ (FP, IP and TP, respectively) [16-18]. IP, DP₁, EP₂ and EP₃ receptors are coupled with the activation of Gs proteins and linked to increases in intracellular cAMP, whereas EP₁, FP and TP signal through Gq-mediated increases in intracellular calcium concentration. Exceptionally, the EP₁ receptor is coupled to Gi and decreases cAMP formation. Interestingly, in addition to signaling through G-protein linked receptors, PGs generated from COX-2 located in the nuclear envelope may mediate signals preferentially through a nuclear pathway, whereas COX-1 derived PGs may mediate signals solely through cell surface receptors [16]. This emphasizes the importance of COX-2 and nuclear receptors (i.e., peroxisome proliferator-activating receptors, PPARs) in cell growth and survival.

**COX ISOZYMES: COX-1, COX-2 AND NOW COX-3**

The understanding that NSAIDs block prostanooid formation via inhibition of COX explained the anti-inflammatory effects of these drugs. Similarly, prostanooids, such as PGE₂ and prostacyclin, were found to be protective to the stomach and, therefore, inhibition of their formation provided an explanation for the gastrointestinal toxicity associated with prolonged use of NSAIDs (see later in this review). On the other hand, inhibition of COX in platelets explained the ability of aspirin to reduce platelet aggregation and blood clotting. But still a number of questions remained unanswered; such as why the toxicity of NSAIDs differs in the gastrointestinal tract when used at similar anti-inflammatory doses? The answer was provided in the early 1990’s when it was established that there are at least two COX isozymes present in human cells: COX-1, which is constitutively expressed; and COX-2, which is inducible [19-21].

COX-1 and COX-2 are the products of two distinct genes, which in humans are localized on chromosomes 9 and
The COX-1 gene is about 22 kb in length with 11 exons and is transcribed as a 2.8 kb mRNA [22, 24]. The COX-2 gene is about 8 kb long with 10 exons and it is transcribed as 4.6, 4.0 and 2.8 kb mRNAs variants [25-27]. Sequence analysis of the COX-2 5′-flanking region has revealed several potential transcription regulatory elements, including a TATA box, a NF-IL-6 motif, two AP-2 sites, three Sp1 sites, two NF-kB sites a CRE motif and an E-box [28].
The amino acid sequences of COX-1 and COX-2 from a single species are about 60% identical. COX-1 is a glycoprotein that in its native, processed form has 576 amino acids with an apparent molecular mass of 70 kDa [22, 24]. The cDNA for COX-2 encodes a polypeptide that, before cleavage of the signal sequence, contains 604 amino acids with an apparent molecular mass of 70 kDa [25, 26]. The main difference is that the COX-1 protein contains a 17 amino acid sequence near its amino terminus that is not present in COX-2. In contrast, COX-2 contains a 18 amino acid sequence near its carboxyl terminus that is not present in COX-1 [22, 24-26]. However, and as already mentioned, the two COX isoforms catalyze identical reactions and exhibit the same kinetic constants for the conversion of arachidonic acid to prostanoids.

The most striking distinctions between COX-1 and COX-2 are the differential regulation of their expression and their tissue distribution (Fig. 2). COX-1 is ubiquitous and is constitutively expressed throughout the gastrointestinal system, the kidneys, the vascular smooth muscle and platelets [28]. COX-1 is presumably involved in the housekeeping functions of PGs, such as the cytoprotective effects in the gastric mucosa, the integrity of platelet function and the maintenance of renal perfusion. Conversely, COX-2 is undetectable in most tissues, but its expression can be induced by a variety of stimuli related to inflammatory response. COX-2 is, therefore, commonly referred to as the inducible COX isoform because, like other immediate-early genes, it can be rapidly upregulated in response to growth factors and cytokines [28]. This has led to the supposition that the inducible COX-2 isoform is responsible for the synthesis of PGs involved in inflammatory response, whereas COX-1-derived PGs are involved in preserving the physiological functions of these prostanoids. However, this distinction is not entirely accurate, since COX-1 can be induced or upregulated under certain conditions and COX-2 has been consistently shown to be constitutively expressed in organs, such as the brain and the kidneys (see later in this review).

Although the discovery of COX-2 was a great advancement in our understanding of COX biology, it did not appear to explain everything. In particular, the mechanism of action of acetaminophen remained a mystery. Hugely popular and with a history almost as venerable as that of aspirin, this drug exerts antipyretic and analgesic effects without displaying anti-inflammatory activity. Moreover, at therapeutic concentrations, acetaminophen only weakly inhibits COX-1 and COX-2. Willoughby first suggested that this is because of the presence of another COX isozyme, COX-3 [29]. In fact, Dan Simmons’s group has recently demonstrated the existence of a new COX that is especially sensitive to acetaminophen and related compounds (Fig. 2) [30]. This novel COX, termed COX-3, is a splice variant of COX-1 and in human tissues its mRNA is expressed as an 5.2-kb transcript, which is most abundant in cerebral cortex and heart [30].

**Fig. (2).** Proposed separate functions for prostaglandins (PGs) derived from cyclooxygenase (COX)-1, -2 and -3. While COX-1 is found in most tissues and is believed to participate in physiologic homeostasis in the stomach, kidneys and platelets, COX-2 is preferentially expressed in cells that mediate inflammation and in the central nervous system. Therefore, inhibition of COX-2 is supposed to be responsible for the beneficial actions of non-steroidal anti-inflammatory drugs (NSAIDs), whereas inhibition of COX-1 accounts for the unwanted side-effects of these drugs. A splice variant of COX-1, termed COX-3, that is especially sensitive to acetaminophen and may explain the antipyretic and analgesic effects of this drug, has been recently identified.
INHIBITION OF THE COX PATHWAY: NSAIDS AND COXIBS

COX is the main pharmacological target for NSAIDs. The observation that NSAIDs reduce or prevent the production of PGs by direct inhibition of COX enzymes was first reported by Vane, Ferreira et al. and Smith et al. in 1971 [5-7]. At present, NSAIDs are among the most widely prescribed class of pharmaceutical agents worldwide, having broad clinical utility in treating pain, fever and inflammation [31, 32]. The most popular NSAID, aspirin, is also sought as a potentially viable option in the prevention of sporadic colon cancer and neurodegenerative disorders [33, 34].

Unfortunately and apart from the beneficial anti-inflammatory, antipyretic and analgesic effects of NSAIDs, COX inhibition also results in unwanted side effects, particularly in the gastrointestinal tract [35]. Gastroduodenal ulceration is the best-characterized serious adverse event of NSAID therapy and is the consequence of inhibiting PGs, which are the most important gastric cytoprotective agents [36]. On the other hand although the incidence of renal side effects in healthy subjects is not significant, adverse renal events are frequent in those patients with impaired effective arterial blood volume in which renal function is critically dependent on PGs, such as decompensated liver cirrhosis [37]. Since COX-1-derived PGs are presumably involved in housekeeping functions, such as gastrointestinal cytoprotection, and COX-2-derived PGs are implicated in inflammation, NSAID gastrotoxicity is considered to be the consequence of the inhibition of both COX-1 and COX-2 isozymes by traditional NSAIDs (i.e., aspirin, indomethacin, ibuprofen, meclofenamate, …). That is, at the concentrations required to inhibit PG biosynthesis at sites of inflammation (COX-2 activity), they also elicit a marked suppression of PG production in the gastrointestinal and renal systems (COX-1 activity) jeopardizing the integrity of the gastric mucosa and renal and platelet function.

The discovery of the COX-2 isozyme and the characterization of its role in inflammation fostered the development of a new class of compounds that selectively inhibit COX-2, without affecting the COX-1-dependent PG biosynthesis necessary for physiological functions [38-41]. This new generation of anti-inflammatory drugs has been proven in vitro to selectively inhibit COX-2 activity and to be as efficacious as standard NSAIDs in a number of in vivo models of inflammation (rat carrageenan-induced foot pad edema and rat adjuvant-induced arthritis) and hyperalgesia (rat carrageenan-induced hyperalgesia) [40, 41]. Two COXIBs (selective COX-2 inhibitors), celecoxib (Celebrex®) and rofecoxib (Vioxx®) have been marketed in the past three years, and in clinical trials have proven to provide significant relief of the signs and symptoms of osteoarthritis and rheumatoid arthritis and in alleviating pain following dental extraction, while reducing the incidence of gastrointestinal ulcers and erosions seen with standard NSAID therapy [42-47]. Moreover, these eagerly awaited highly selective COX-2 inhibitors are of great interest because they may represent an alternative therapeutic option for the treatment of inflammation in diseases, such as in cirrhosis with ascites, in which renal function is critically dependent on PGs [48, 49]. A second-generation of selective COX-2 inhibitors (i.e., valdecoxib and etoricoxib) with a higher COX-1 to COX-2 selectivity ratio than celecoxib and rofecoxib is currently under evaluation in patients with osteoarthritis and rheumatoid arthritis. Furthermore, the analgesic efficacy and tolerability of parecoxib, an injectable prodrug of valdecoxib, is currently being tested in postoperative laparotomy and orthopedic knee surgery patients [50, 51]. A list of currently available NSAIDs and COXIBs is provided in Table 1.

COX-2 IN HEALTH AND DISEASE

Activation of prostanoid receptors triggers an astonishing array of biological effects (Table 2). The most important targets of PGs include the gastrointestinal tract, the

Table 1. Classification of Non-Steroidal Anti-Inflammatory Drugs According to their Selectivity Towards COX-2. Brand Names are Given in Brackets

<table>
<thead>
<tr>
<th>Traditional NSAIDs</th>
<th>Preferential COX-2 inhibitors</th>
<th>Selective COX-2 inhibitors</th>
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<tbody>
<tr>
<td>Aspirin</td>
<td>Etodolac (Lodine)</td>
<td>Celecoxib (Celebrex)</td>
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<tr>
<td>Diclofenac (Arthrotec, Cataflam, Voltaren)</td>
<td>Meloxicam (Mobic)</td>
<td>Etoricoxib (Arcoxia)</td>
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<tr>
<td>Flurbiprofen (Ansaid)</td>
<td>Nabumetone (6-MNA) (Relafen)</td>
<td>Parecoxib (Dynastat)</td>
</tr>
<tr>
<td>Ibuprofen (Advil, Motrin, Naprin, Others)</td>
<td>Nimesulide (Mesulid, Nexen)</td>
<td>Rofecoxib (Vioxx)</td>
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<tr>
<td>Indomethacin (Indocin, Inacid, Others)</td>
<td></td>
<td>Valdecoxib (Bextra)</td>
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<tr>
<td>Ketoprofen (Orudis, Oruvail)</td>
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<tr>
<td>Ketorolac (Acular, Toradon)</td>
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<tr>
<td>Naproxen (Aleve, Naprelan, Naprosyn)</td>
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<td>Piroxicam (Feldene)</td>
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<td>Sulindac (Clinoril)</td>
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<td>Tolmetin (Tolectin)</td>
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cardiovascular system, the central and peripheral nervous systems, the renal system and the reproductive organs. A detailed discussion of PG actions and the role of COX-2 under physiologic and pathologic conditions is herewith provided.

**COX-2 and the Gastrointestinal Tract**

**Gastric Secretion**

In human gastric mucosa, PGE\(_2\) and PGF\(_{2\alpha}\) have been reported as the most abundant COX-derived products, with low production of PGD\(_2\) and PGI\(_2\) [52]. In 1967, initial observations reported PGE\(_2\) to be anti-secretory and anti-ulcerogenic [53]. Subsequently, it was demonstrated that intravenous administration of PGE\(_1\) or PGE\(_2\) inhibits acid and pepsin secretion induced by secretagogues, such as pentagastatin and histamine [54]. Moreover, PGE\(_2\) has been shown to inhibit gastric parietal cells and to increase bicarbonate secretion, resulting in decreased acidity of gastric juice [55]. It must be taken into account that these observations on gastric acid secretion were obtained by administering PGs at pharmacological doses and that establishing the role of endogenously produced PGs in gastric acid secretion has proven to be more challenging. In this regard, blockade of PG biosynthesis with COX inhibitors has yielded disparate and non-conclusive results [56]. Moreover, although studies in experimental models have shown that animals actively or passively immunized with PG-protein conjugates develop gastrointestinal tract erosions and ulcerations primarily in the stomach and occasionally in the small bowel, in these experiments basal and stimulated acid secretion did not differ in the treated versus non-treated animals [57]. Therefore, the role of endogenous COX-derived products in gastric secretory physiology still remains controversial.

Recently, COX-2 has been reported to be induced in human gastric mucosa infected with *Helicobacter pylori* [58, 59]. Moreover, the production of PGE\(_2\) in gastric mucosa is increased in patients with gastritis and infection with *H. pylori* stimulates synthesis of PGE\(_2\) in gastric mucosal cells *in vitro* [58, 60]. While *H. pylori* infection is considered to increase acid secretion in duodenal ulcer patients, in severe gastritis, acid secretion is generally decreased and basal gastrin levels are assumed to be stimulated. In particular, acid secretion was significantly reduced in an experimental group of mice with *H. pylori* infection, an effect that was restored to the same level as in the control mice following COX-2 inhibition [61].

**Gastrointestinal Motility**

Essential to digestion, gastrointestinal motility is a complex process regulated by both neural and humoral components. Paracrine modulation of normal intestinal motility by eicosanoids such as PGs is still a matter of debate, but pharmacological doses of certain prostanooids have been shown to alter motility both *in vivo* and *in vitro* [62]. Vascular reactivity studies in isolated intestinal muscle show that PGE\(_2\) induces contraction of longitudinal muscle and relaxation of circular muscle, whereas PGF\(_{2\alpha}\) induces contraction of both muscles [63]. Isolated colonic longitudinal and circular muscle layers respond to PGE\(_2\) and PGF\(_{2\alpha}\) in the same manner as the small intestine, although the results obtained preclude any generalization. For example, low doses of PGE\(_2\) can induce activity in the distal colon and be inhibitory in the proximal colon [64]. On the other hand, in most animal studies, PGs of the E series relax the lower esophageal sphincter whereas PGF\(_{2\alpha}\) constricts it [55]. In any case, when administered *in vivo* both PGs decrease transit time and produce diarrhea through increased

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**Table 2. Biological Effects of Cyclooxygenase Products**

<table>
<thead>
<tr>
<th>Tissue/Organ</th>
<th>Mediators</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female Reproductive Organs</td>
<td>PGE(<em>2), PGF(</em>{2\alpha})</td>
<td>Uterine Contraction, Oxytocic Action</td>
</tr>
<tr>
<td>Male Reproductive Organs</td>
<td>PGE(<em>2), PGF(</em>{2\alpha})</td>
<td>Fertility</td>
</tr>
<tr>
<td>Cardiovascular System</td>
<td>TXA(_2), PGI(_2) TXA(_2) PGE(_2), PGI(_2) TXA(<em>2), PGE(</em>{2\alpha}) PGE(_2), PGI(_2)</td>
<td>Thrombosis, Platelet Aggregation</td>
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<td></td>
<td></td>
<td>Vascular Permeability</td>
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<td></td>
<td></td>
<td>Arterial Vasodilation</td>
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<tr>
<td></td>
<td></td>
<td>Venous vasoconstriction</td>
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<tr>
<td></td>
<td></td>
<td>Patency of the Fetal Ductus Arteriosus</td>
</tr>
<tr>
<td>Respiratory System</td>
<td>PGE(<em>2) PGE(</em>{2\alpha})/TXA(_2)</td>
<td>Bronchodilation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bronchoconstriction</td>
</tr>
<tr>
<td>Renal System</td>
<td>PGE(_2), PGI(_2) PGE(_2), PGI(_2) PGE(_2)</td>
<td>Regulation Renal Blood Flow and Glomerular Filtration Rate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Renin Release</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibition Hydroosmotic Effect of ADH</td>
</tr>
<tr>
<td>Gastrointestinal System</td>
<td>PGE(_2), PGI(_2)</td>
<td>Cytoprotection</td>
</tr>
<tr>
<td>Immune System</td>
<td>PGE(_2), PGI(_2)</td>
<td>Inhibition T and B lymphocyte activation and proliferation</td>
</tr>
<tr>
<td>Central Nervous System</td>
<td>PGE(_2), PGI(_2)</td>
<td>Fever</td>
</tr>
<tr>
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<td>Sleep</td>
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<tr>
<td></td>
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<td>Pain</td>
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Little is known about the roles that COX-1 and COX-2 may play in the regulation of gastrointestinal motility. Although several studies have shown that the non-selective NSAIDs indomethacin and aspirin impair intestinal peristalsis, which is the most relevant clinical motor pattern of the gut, recent findings suggest that this effect is unrelated to COX inhibition and have different targets, such as nuclear factor-κ B and/or peroxisome proliferator-activated receptors γ [67]. In fact, the selective COX-2 inhibitor NS-398 has been shown not to affect gastric motility in rats with gastroduodenal ulcerogenic responses induced by hypothermic stress [68]. Nevertheless, a role for prostanooids derived from COX-2 in postoperative bowel motility has been proposed. In this regard, selective inhibition of COX-2 significantly increases postoperative small bowel transit in experimental models of postoperative ileus [69, 70].

**Gastrointestinal Inflammation**

Eicosanoids along with many other soluble factors, such as cytokines, reactive oxygen species, bacterial products and lipid mediators, play a critical role in gastrointestinal inflammation. Several eicosanoids, including products from COX (PGE$_2$ and TXB$_2$) and lipoxygenase (leukotriene (LT) B$_4$ and HETEs), are increased in inflamed tissues compared with normal mucosa [71]. A positive correlation exists between the increase in these eicosanoids and the activity of the disease, returning the levels during remission to values similar to those from normal colorectal mucosa [55]. An increasing body of evidence, especially in animal models of inflammatory bowel disease (IBD), indicate that PGs counteract the pro-inflammatory activity of LTB$_4$. Indeed, the administration of NSAIDs to either patients or animal models with IBD does not have a salutary effect but rather is associated with flare-ups and colitis [72, 73]. This phenomenon is interpreted as NSAIDs suppressing PGE$_2$ formation thus allowing LTB$_4$ synthesis to proceed toward pro-inflammation.

In experiments using animal models of inflammation, such as carrageenan injection into the footpad and the murine air pouch model, COX-2 expression and activity is markedly upregulated [38, 74]. In these experimental models of acute inflammation, selective COX-2 inhibition by inhibiting edema at the inflammatory site and being analgesic is beneficial [38, 74, 75]. Therefore, because there is increased expression of COX-2 in both animal models of colitis and in human IBD [76, 77], in theory, selective COX-2 inhibition would also likely be beneficial in this disease. Although preliminary results suggest that COX-2 inhibitors may be safe and beneficial in most patients with IBD [78], other investigators do not agree because they have enough evidence to support that PGs derived from COX-2 are important in promoting the healing of mucosal injury, in protecting against bacterial invasion, and in down-regulating the mucosal immune system [79]. Indeed, in experimental models, suppression of COX-2 in a setting of gastrointestinal inflammation and ulceration has been shown to result in the impairment of healing and exacerbation on inflammation-mediated injury [76, 79]. In humans, clear cases of exacerbation of IBD have been reported with the use of a selective COX-2 inhibitor [80]. Therefore, the use of COX-2 selective inhibitors in patients with IBD should be viewed with the same caution as the use of traditional NSAIDs.

**COX-2 and Colon Cancer**

The most solid, unequivocal and well-established role of COX-2 in the gastrointestinal system is its participation in colon cancer [81-84].

Early observations in this field were obtained in studies comparing the levels of PGs in colon tumors with those of normal colon tissue. The concentration of PGs, in particular of PGE$_2$, was found increased in human colorectal cancer tissue when compared with normal colonic mucosa [85-87]. Similar findings were reported in experimental models of colonic carcinogenesis [88]. These early investigations also established which PG series is involved in colon cancer. Rigas et al. compared the levels of different PGs in colon tumors with those of normal colon tissue and found that PGE$_2$ was elevated about two-fold in the cancer samples [86]. In contrast, no significant changes were found in the levels of PGF$_2$ and TXA$_2$, and, even, PGI$_2$ was reduced about three-fold in tumors [86]. Therefore, because both human and experimental colonic tumors produce increased amounts of this particular PG, PGE$_2$ appears to be the eicosanoid involved in colon carcinogenesis.

Since PGE$_2$ levels are increased in tumors, COX is suspected to play a key role in the progression of colon cancer. The relative levels of COX-1 and COX-2 expression in colorectal cancers were evaluated by a number of independent laboratories. All the investigations reported increased expression of COX-2, but not COX-1, in colorectal carcinomas [89-91]. Published reports indicate that roughly 85% of adenocarcinomas exhibit a two- to fifty-fold increase in COX-2 expression at both mRNA and protein levels compared with matched, macroscopically normal, colonic mucosa from the same patient [89-92]. Both carcinogen-induced (azoxymethane-induced colonic tumors in rats) and genetic (Min mice with multiple intestinal neoplasia) animal models of colon cancer have confirmed the existence of an increased expression of COX-2 in tumors [92-94]. Nevertheless, although COX-2 is differentially expressed in various regions of the colon in human colorectal cancer, with the COX-2 protein being greatly over-expressed in tumors located in the rectum [89-92], at present its cellular origin is still debated. Cancer cells, nontransformed epithelial cells, stromal cells, vascular endothelial cells and inflammatory infiltrates are constituents of colon tissue and all of these cell types are reported to have elevated levels of COX-2 compared with the normal colon tissues [95]. On the contrary, subsequent studies have demonstrated that the increased expression of COX-2 in colon cancer originates mainly from the interstitial cells (i.e., macrophages), whereas little COX-2 expression is found in epithelial cancer cells [96, 97].

Several mechanisms have been suggested for the enhanced COX-2 gene expression in colon cancer, including mutations of APC and ras, activation of EGF receptor and IGF-1 receptor pathways and heresulin/HER-2 receptor pathway, direct induction by the Epstein-Barr virus oncprotein and latent membrane protein 1 [98-100]. For
instance, Oshima et al. assessed the development of intestinal adenomas in APC316 knockout mice (a model in which a targeted truncation deletion in the tumor suppressor gene APC causes intestinal polyposis) in a wild type and homozygous null COX-2 genetic background. The number and size of polyps were dramatically reduced (six to eight-fold) in the COX-2 null mice compared with COX-2 wild-type mice [101].

In vitro, the functional consequences of COX-2 overexpression have been analyzed in the rat intestinal cell line (RIE cells), the gastrocolonic cell lines HT-29 and HCA-7 and in the human colon cell line Caco-2 transfected with COX-2. Overall, these experiments have revealed that cells over-expressing COX-2 undergo phenotypic changes that could enhance their tumorigenic potential, such as acquisition of an increased adhesion to extracellular matrix proteins and resistance to apoptosis [102, 103]. The proliferative activity of COX-2 is believed to be primarily mediated by PGs. Along these lines, the human gastrocolonic cancer cell line, HT-29, shows increased proliferation in the presence of PGs in the culture medium [104]. Moreover, in vivo, colonicocyte proliferation is significantly stimulated by the injection of the stable derivative of PGE2, dimethyl PGE2, into normal mice [104]. A conclusive proof of the role of COX-2 in cell growth is provided by the use of selective COX-2 inhibitors. The effects of the highly selective COX-2 inhibitor, SC-58125, was tested in two different cell lines, only one of which had a high level of COX-2 expression and activity. It was observed that SC-58125 decreased cell growth only in the COX-2 expressing cell line [105].

In vivo, in human and experimental animals, a body of evidence support the concept that inhibition of COX, and, in particular COX-2, protects against colon cancer. Epidemiological studies have demonstrated the association between regular long-term consumption of NSAIDs, in particular aspirin, and reduced incidence of colon cancer [33, 106-108]. Aspirin and sulindac have also been shown to reduce the number and size of its nonmalignant precursor, the adenomatous colonic polyp, in patients with familial adenomatous polyposis (FAP) [109, 110]. Moreover, a recent paper provides evidence of a protective association between aspirin and NSAIDs and esophageal cancer [111]. These epidemiological data are considered robust, because separate studies differing in locale, design and population have consistently yielded similar results. Parallel studies in animal models of colon carcinogenesis have also proven that aspirin, as well as other traditional NSAIDs, such as piroxicam, indomethacin, sulindac, ibuprofen and ketoprofen, inhibit chemically-induced colon cancer in rats and mice [33]. The mechanism by which NSAIDs reduce the risk of cancer is likely related to the inhibition of COX-2. In fact, a randomized clinical trial has shown that the selective COX-2 inhibitor, celecoxib, effectively inhibits the growth of adenomatous polyps and causes regression of existing polyps in patients with hereditary FAP [112]. Studies in rodents have also demonstrated that selective pharmacological inhibition of COX-2 activity prevents chemically-induced carcinogenesis and intestinal polyp formation in an experimental model of FAP [113-115]. In Min mice and rats exposed to chemical carcinogens, selective COX-2 inhibitors induce apoptosis as well as stimulate apoptosis and suppress growth in many carcinomas, including cultured human cancers of the stomach, esophagus, tongue, brain, lung and pancreas [116].

Recent studies indicate that angiogenesis, which is the development of new blood vessels and an essential step in tumor growth, requires COX-2 [84]. In fact, COX-2 overexpressing cells produce large amounts of vascular endothelial growth factor (VEGF), a key proangiogenic factor, which stimulates both endothelial migration and in vitro angiogenesis [102]. These effects may be blocked by selective COX-2 inhibitors, highlighting the potential application of these compounds in blocking developing tumors [113]. On the other hand, mice lacking the COX-2 gene have deficient VEGF expression, reduced tumor angiogenesis and decreased tumor growth [117]. Moreover, selective inhibition of COX-2, but not COX-1, suppresses the growth of corneal capillary blood vessels in rats exposed to basic fibroblast growth factor and inhibits the growth of several human tumors implanted in mice [117, 118]. Taken together, these results support the notion of the benefit of selective COX-2 inhibition as an anti-angiogenic therapy.

In addition to the well-documented implication of COX-2 in colon cancer, several investigations have recently examined its potential role in other epithelial cancers. These studies have provided strong evidence that COX-2 may also be implicated in breast, head and neck, lung, pancreatic and gastric cancers and suggest that the beneficial effects of COX-2 inhibitors may extend to these other cancers [119-122]. Indeed, celecoxib consistently and dose-dependently inhibits tumor growth and the number of lung metastasis in the syngenic Lewis lung carcinoma model [116]. Interestingly, in animal studies, celecoxib has been shown to potentiate the antitumor activity of conventional chemotherapy and radiation [123, 124].

**COX-2 and the Kidney**

PGs play multiple roles in the adult kidney. PGE2 and PGI2 are powerful renal vasodilators that modulate intrarenal vascular tone and influence glomerular hemodynamics and renal perfusion [125, 126]. Moreover, PGE2 modulates renal sodium through direct inhibition of sodium reabsorption in tubular epithelial cells [125, 126]. PGE2 also modulates renal water homeostasis by antagonizing the hydroosmotic effect of antidiuretic hormone in the collecting duct [125, 126]. Finally, PGs are involved in the renal responses to loop diuretics and in the regulation of renin secretion [126, 127]. Since PG synthesis is initiated by COX-1 and COX-2, and both COX isoforms are constitutively expressed in the kidneys [128, 129], it was of interest to establish which COX isoform is involved in the maintenance of renal function under normal conditions. The distribution of COX-1 in the renal area differs significantly from that of COX-2. In this regard, COX-1 is preferentially expressed in the renal vasculature and in the medullary and papillary collecting ducts in humans, monkeys, dogs, rabbits and rats, whereas COX-2 expression is consistently focal, and limited to the macula densa of the juxtaglomerular apparatus, epithelial cells of the thick ascending limb and papillary interstitial cells of rats, rabbits and dogs [128, 129]. In the adult human kidney, expression of COX-2 protein has been observed in
endothelial and smooth muscle cells of arteries and veins, and intra-glomerularly in podocytes, but not in macula densa cells [130].

At present, it is hypothesized that only PGs derived from COX-2 are involved in normal renal function. The marketed selective COX-2 inhibitors, rofecoxib and celecoxib, have been evaluated in randomized controlled trials in terms of their effects on renal PGs, renal function and occurrence of adverse renal events. Most of the studies have reported that selective COX-2 inhibition does not significantly impair renal function in healthy subjects [131]. Nevertheless, it is worthy to note that in healthy elderly patients, a population more susceptible to renal NSAID-damaging effects, both celecoxib and rofecoxib induce a reduction in urinary sodium excretion similar to that of indomethacin and naproxen [132, 133]. Although the role of COX-2-derived PGs in the kidney is still under debate, the finding that mice lacking the COX-2 gene develop mild to severe nephropathy during the first weeks after birth [134, 135], supports the eventuality that COX-2-derived PGs may play a physiological role in the kidney other than maintenance of circulatory and excretory functions.

The incidence of renal side effects secondary to NSAID treatment is low, and the role of PGs in renal homeostasis is hardly noticeable in healthy subjects. In contrast, administration of NSAIDs to patients with unbalanced effective arterial blood volume represents a major clinical problem since renal function in these patients is critically dependent on PGs [37]. In these circumstances, which include low sodium diet, hemorrhagic hypovolemia and edematous conditions associated with congestive heart failure, nephrotic syndrome and decompensated liver cirrhosis, NSAIDs induce a pronounced impairment in renal plasma flow, glomerular filtration rate, urine volume and urinary sodium excretion. Since selective COX-2 inhibitors have been developed to effectively inhibit COX-2 while sparing physiologic PG production from COX-1, these novel compounds could potentially be the anti-inflammatory of choice in these patients. In this regard, in recent studies in rats with carbon tetrachloride-induced cirrhosis and ascites, an experimental model that closely reproduces human liver disease, selective COX-2 inhibition with celecoxib did not seriously compromise renal function [48, 49]. Interestingly, in these animals COX-1 protein expression was found to be unchanged whereas COX-2 protein levels were consistently upregulated [49]. Therefore, these results indicate that despite abundant renal COX-2 protein expression, the maintenance of renal function in altered effective arterial blood volume conditions, such as liver cirrhosis is mainly dependent on COX-1-derived PGs.

**COX-2 and the Brain**

PGs have long been recognized as mediators of fever. Fever is produced by PGE$_2$, acting on temperature-sensing neurons in the preoptic area [136]. The endogenous fever-producing PGE$_2$ is thought to originate from COX-2 induced by lipopolysaccharide or interleukin-1 in endothelial cells lining the cerebral blood vessels [137]. In fact, intraperitoneal injection of lipopolysaccharide to rats induces COX-2 in brain endothelial cells in parallel with the appearance of fever [138]. Moreover, selective inhibitors of COX-2 are potent antipyretic agents [139].

PGs are also now recognized as mediators of inflammatory reactions in neural tissue and more recently of brain function. Constitutive COX-2 immunoreactivity and COX-2 mRNA expression have been detected in neurons and specially in the forebrain [140]. The expression of COX-2 in the brain is particularly high in neonates [141]. Since brains of neonates have higher levels of cerebral PGs than those of adults [141], and since these PGs are involved in the regulation of blood flow in the newborn [142], this phenomenon suggests that COX-2 is likely to be important in the modulation of blood flow at this stage of life. Furthermore, COX-2 is thought to play a key role in the final stages of development and brain modeling when COX-2 becomes active in a manner that coincides with the imprinting of environmental influences [143].

Intense nerve stimulation, leading to seizures, induces COX-2 mRNA in discrete neurons of the hippocampus, whereas acute stress raises levels in the cerebral cortex [140, 144]. The actual role of COX-2 and PGs in these sites is not understood, but associations between COX-2 induction and neural degeneration after glutamate stimulation, seizures and spreading depression waves suggest that COX-2 may play more of a role in the selective loss of neural connections than in their formation [145]. Finally, current studies show that COX-2 potentiates brain parenchymal amyloid plaque formation leading to Alzheimer’s disease, thus supporting a therapeutic potential for NSAIDs and COXIBs in the treatment of this neurological disease [146].

**COX-2 and the Reproductive Function**

PGs are important for inducing uterine contractions during labor. NSAIDs, such as indomethacin, delay premature labor by inhibiting the production of PGs [147]. However, the inhibition of PGs with indomethacin led to a high mortality among infants because of premature closure of the ductus arteriosus, a circulatory shunt maintained by PGs that allows the output of the left ventricle to bypass the fetal lungs [147, 148]. PGs originating from COX-2 may play a role in the birth process because COX-2 mRNA in the amnion and placenta increases markedly immediately before and after the initiation of labor [149]. Theoretically, selective inhibitors of COX-2 are able to reduce PG synthesis in fetal membranes and could be useful in delaying premature labor without the unwanted side effects of traditional NSAIDs. However, COX-2 also plays a role in ovulation, the successful rupture of the follicle and in the implantation of the embryo in the uterine endometrium [150]. Indeed, COX-2 null mice show multiple failures in reproductive function, including ovulation, fertilization, implantation and decidualization [151].

**COX-2 and Inflammation and Arthritis**

The most important action of PGs is their role in inflammation [4]. In response to an inflammatory insult, the release of PGs, and more importantly PGE$_2$, constitutes a key event in the development of the three cardinal signs of inflammation: swelling/redness, pain and fever. Given its
potent vasodilatory properties. PGE$_2$ increases tissue blood flow, which results in the characteristic erythema (redness) of inflammation [4]. On the other hand, together with other soluble factors including bradykinin, histamine and leukotrienes, PGE$_2$ increases vascular permeability contributing to fluid extravasation and the appearance of edema (swelling) [4]. PGE$_2$ also sensitizes afferent nerve fibers acting both at peripheral sensory neurons and at central sites within the spinal cord and brain to evoke hyperalgesia (pain) [4]. Finally PGE$_2$ is a potent pyretic mediator involved in the appearance of fever [136]. Moreover, PGs also contribute to the amplification of the inflammatory response by enhancing and prolonging signals produced by pro-inflammatory agents, such as bradykinin, histamine, neurokinins and complement.

Although the importance of COX and PGs in inflammation has been known for years, the inducibility of COX and PG formation has only fully been appreciated recently with the uncovering of the properties of COX-2. COX-2 was originally discovered as a transcript that was upregulated during inflammation and cellular transformation [19-21]. Animal models of inflammatory arthritis provided the first available information regarding expression of COX-2 in acute and chronic inflammation, indicating that increased expression of COX-2 is responsible for the increased PG production seen in inflamed joint tissues [75]. COX-2 induction was observed in both human osteoarthritics-affected cartilage as well as in synovial tissue taken from patients with rheumatoid arthritis [152, 153]. The critical role of COX-2 in inflammation led to the rational design of the first clinical trials for selective COX-2 inhibitors in patients with osteoarthritis and rheumatoid arthritis [42-45]. The results of these drug-screening studies are sufficient to guarantee that COX-2 inhibitors achieve the same anti-inflammatory efficacy as traditional NSAIDs [42-45].

**COX-2 and the Cardiovascular System**

The major effects of PGs on the cardiovascular system are on the smooth muscle cells and platelets where prostacyclin (PGI$_2$) has opposite biologic properties to those of TXA$_2$. Thus, on the vasculature and especially on veins, PGI$_2$ mainly promotes vasorelaxation while TXA$_2$ acts as a vasoconstrictor [4]. It was classically considered that PGI$_2$ produced by vascular endothelial and smooth muscle cells was formed via COX-1. However, recent work has demonstrated that PGI$_2$ in vascular cells is produced by COX-2 as well as COX-1 [28]. Treatment of volunteers with a selective COX-2 inhibitor decreased urinary excretion of PGI$_2$ without affecting TXA$_2$ [132]. These results raised the possibility of an increased risk of cardiovascular events associated with COXIBs. However, two major randomized trials have recently shown that the relative risk of a cardiovascular event with selective COX-2 inhibitors is similar to that of traditional NSAIDs [154, 155]. On the other hand, in platelets, TXA$_2$ induces platelet aggregation whereas PGI$_2$ strongly inhibits aggregation. It is widely recognized that platelets express only COX-1. Nevertheless, in certain stages of megakaryogenesis, COX-2, as well as COX-1 are detected [156]. The biological significance of this phenomenon is, at present, unclear.

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
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<td>COXIBs</td>
<td>Selective COX-2 inhibitors</td>
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<td>FAP</td>
<td>Familial adenomatous polyposis</td>
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<td>LT</td>
<td>Leukotrienes</td>
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<td>NSAIDs</td>
<td>Non-steroidal anti-inflammatory drugs</td>
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<td>PGs</td>
<td>Prostaglandins</td>
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<td>VEGF</td>
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**REFERENCES**

References 157-159 are related articles recently published in Current Pharmaceutical Design.


Cyclooxygenase-2 Biology


