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Cyclooxygenase isoforms and atherosclerosis

Orina Belton and Desmond J. Fitzgerald

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of arthritis and pain. However, their long-term use is limited by gastrointestinal (GI) side effects such as gastric ulcers. NSAIDs act by inhibiting an enzyme called cyclooxygenase. Cyclooxygenase (COX) catalyses the generation of prostaglandins from arachidonic acid. Two isoforms of the enzyme exist – COX-1 and COX-2 – both of which are targets for NSAIDs. Although they are associated with GI toxicity, NSAIDs have important antithrombotic and anti-inflammatory effects. The GI injury has been attributed to COX-1 inhibition and the anti-inflammatory effects to COX-2 inhibition. As COX-2 is traditionally viewed as an inducible enzyme, selective inhibition of COX-2 by 'coxibs' (selective COX-2 inhibitors) has been employed to achieve anti-inflammatory and analgesic effects without GI side effects. However, recently there have been suggestions that chronic administration of coxibs might increase the risk of cardiovascular events, such as atherosclerosis, compared with traditional NSAIDs. In vascular disease, there is increased expression of both COX-1 and COX-2, resulting in enhanced prostaglandin generation. The specific role of COX-1 and COX-2 in vascular regulation is still unknown but such knowledge is essential for the effective use of coxibs. Although more evidence is pointing to selective COX-1 inhibition as a therapeutic measure in inflammatory atherosclerosis, there are some studies that suggest that inhibition of COX-2 might have a potential benefit on atherosclerosis.

Atherosclerosis, manifested by heart disease, stroke and peripheral vascular disease, has been described as an inflammatory disease (Ref. 1) characterised by mononuclear infiltration and smooth muscle cell proliferation (Ref. 2). As at other sites of inflammation, eicosanoid generation is enhanced in atherosclerosis (Ref. 3). The products include thromboxane A_2 (TXA_2), which

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is a potent platelet aggregator, vasoconstrictor, smooth muscle cell mitogen (Ref. 4) and regulator of angiogenesis (Ref. 5), and prostacyclin (PGI_2), which is a platelet inhibitor, vasodilator (Ref. 6) and inflammatory mediator (Ref. 7). Prostaglandins and thromboxane are derived from arachidonic acid by the enzyme prostaglandin synthase, or cyclooxygenase (COX) (Fig. 1). Two isoforms of COX have been identified – COX-1 and COX-2 – which catalyse identical reactions. Both isoforms are targets of nonsteroidal anti-inflammatory drugs (NSAIDs) (Ref. 8). NSAIDs including aspirin have proven anti-inflammatory, analgesic and antithrombotic properties but are associated with gastrointestinal (GI) toxicity. The GI injury has been attributed to COX-1 inhibition and the anti-inflammatory effects to COX-2 inhibition (Ref. 9), although recent evidence suggests that inhibition of both isoforms is necessary for the gastric damage to occur (Ref. 10). As COX-2 is traditionally viewed as an inducible enzyme, selective inhibition of COX-2 (by selective COX-2 inhibitors called coxibs) has been employed to achieve anti-inflammatory and analgesic effects without GI side effects (Ref. 11). However, compared with traditional NSAIDs, chronic administration of coxibs might increase the risk of cardiovascular events such as myocardial infarction and atherosclerosis (Refs 12, 13, 14). This review focuses on the differential role of COX-1 and COX-2 in the vascular system and addresses the potential risks of coxibs in vascular disease.

Overview of COX-1 and COX-2

Membrane phospholipids are enriched with arachidonic acid, which is liberated from cell membranes by the action of phospholipases such as phospholipase A_2 (PLA_2), PLC and PLD in response to stimuli such as histamine (Ref. 15), platelet-derived growth factor (PDGF) (Ref. 16) and interleukin 1 (IL-1) (Ref. 17). The membrane-associated COX enzymes then catalyse the rate-limiting generation of prostaglandins and thromboxane from arachidonic acid. COX-1 is constitutively expressed under physiological conditions (Ref. 18), although there is also evidence that it might be induced particularly at sites of inflammation (Ref. 19). COX-2 is constitutively expressed in some tissues, such as brain (Ref. 20) and kidney (Ref. 21), under normal conditions. However, its expression is induced in response to cytokines (Ref. 22), growth factors (Ref. 23), hypoxia (Ref. 24) and free radicals (Ref. 25) – all of which are factors implicated in the development of atherosclerosis (Ref. 1). COX-1 and COX-2 both catalyse the same two reactions: a cyclooxygenase reaction, in which arachidonic acid is converted to prostaglandin G_2 (PGG_2); and a peroxidase reaction, where PGG_2 undergoes a two-electron reduction to PGH_2 (Ref. 26) (Fig. 1). PGH_2 serves as a substrate for cell-specific isomerases and synthases to produce the prostaglandins and thromboxane (Fig. 1). Although COX-1 and COX-2 catalysis are indistinguishable, the differences in gene and promoter structure, in protein sequence and in subcellular localisation explain the differential regulation of COX-1 and COX-2 in tissues, as described below.

Gene and promoter structure

The genes encoding human COX-1 and COX-2 are located on chromosomes 9 and 11, respectively (Ref. 27). The intron–exon structure of the genes are very similar except that exon 1 and 2 of *COX-1*, which contains the transcription start site and signal peptide, respectively, are condensed into a single exon in *COX-2*. The introns of *COX-2* are smaller than those of *COX-1*, and hence the gene encoding COX-2 is 8 kb and yields a 4.1 kb mRNA (Ref. 27) compared with 22 kb yielding a 2.8 kb mRNA for COX-1 (Refs 28, 29).

COX-1 gene expression is regulated by two promoter regions, located in distal and proximal regions (Ref. 30). It is not clear how the two promoter activities drive the expression of COX-1 under physiological and pathological conditions but it is known that the gene structure of *COX-1* facilitates continuous transcription of a stable message. *COX-1* has no TATA box – rather it has multiple transcription start sites, as is the case for other 'housekeeping' genes (Ref. 26). The human *COX-2* promoter is 1.7 kb in length and has a TATA box that is 31 bp upstream from the transcriptional start site (Ref. 27). Several putative response elements have been identified in the *COX-2* promoter. These include sites for Sp1 and the nuclear factor NF-κB, the cAMP response element (CRE), cGMP response element (GRE), and sites for PEA-3, AP-2 and CAAT-enhancer binding protein (C/EPBβ – also known as NF-IL6) (Ref. 31). The principal response elements proposed to be involved in regulation of *COX-2* gene expression are the NF-κB-binding site and CRE.

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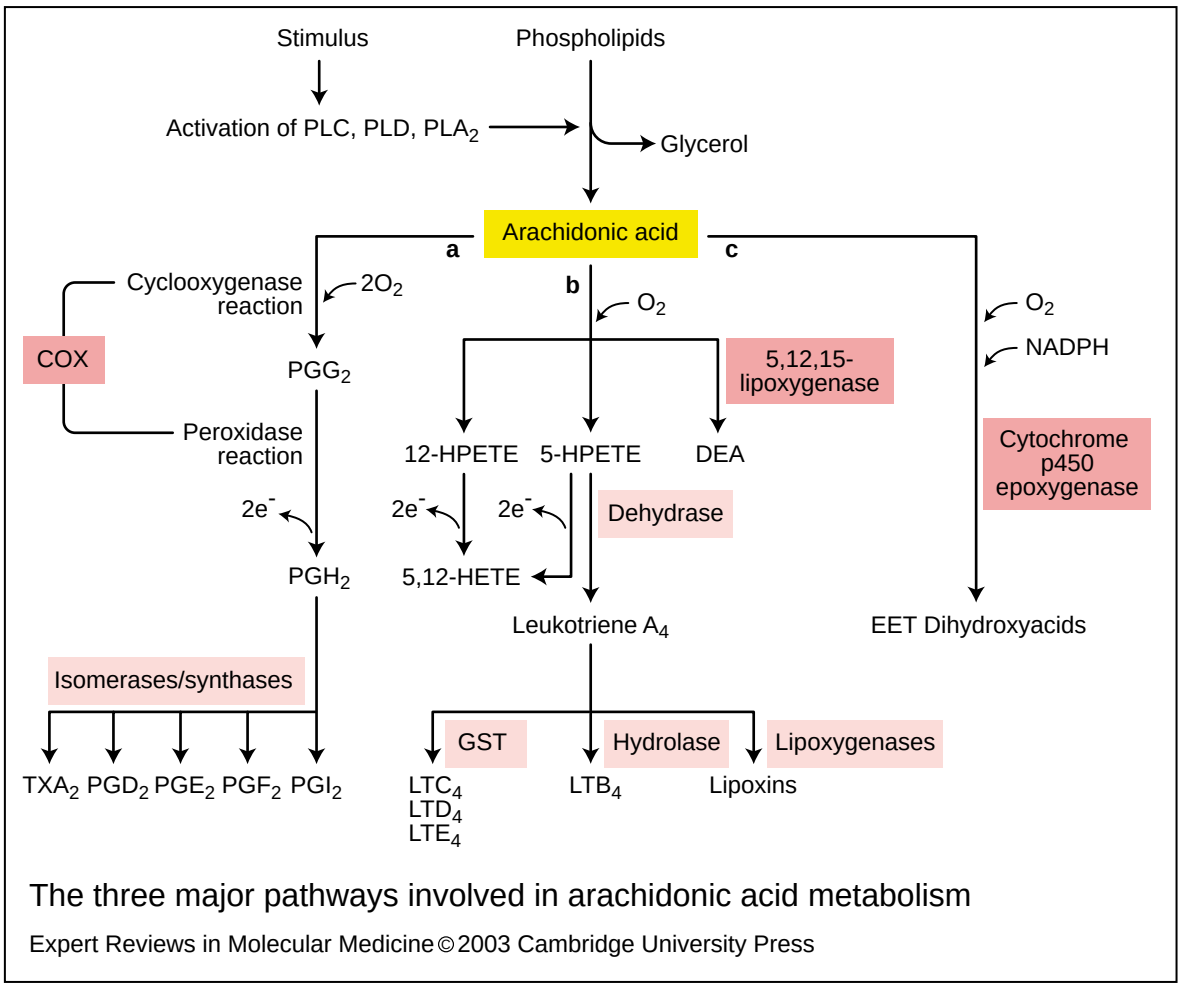

The three major pathways involved in arachidonic acid metabolism

Figure 1. The three major pathways involved in arachidonic acid metabolism. Arachidonic acid is derived directly from linolenic acid or is ingested as a dietary constituent. Arachidonic acid is stored in the cell membrane of virtually all cells and is released in response to stimluli such as histamine and platelet-derived growth factor. Arachidonic acid can be released by three pathways (not shown): (1) conversion of phosphatidyl ethanolamine or phosphatidyl choline to phosphatidic acid in a reaction catalysed by phospholipase D (PLD), followed by formation of diglyceride and monoglyceride and the release of arachidonic acid; (2) degradation of phosphatidylinositol via a sequence of reactions beginning with PLC cleavage of the phosphodiester bond of membrane lipids to yield diacylglycerol, followed by the action of dilglyceride lipase and monoglyceride lipase to release arachidonic acid and glycerol; and (3) direct action of PLA_2 on a phospholipid. (a) The cyclooxygenase (COX) pathway results in the formation of prostaglandin G_2 (PGG_2) from arachidonic acid by a cyclooxygenase reaction. In a subsequent peroxidase reaction, PGG_2 undergoes a two-electron reduction to PGH_2. Both of these reactions are catalysed by COX (prostaglandin synthase H). PGG_2 serves as a substrate for cell-specific isomerases and synthases, producing other eicosanoids such as prostacyclin (PGI_2) and thromboxane A_2 (TXA_2). (b) The lipoxygenase pathway forms hydroperoxyeicosatetraenoic acids (HPETEs) and dihydroxyeicosatetraenoic acid (DEA) by lipoxygenase and subsequently converts these to (1) hydroxyeicosatetraenoic acids (HETEs) by peroxidases, (2) leukotrienes (e.g. LTC_4) by hydrase and glutathione *S*-transferase (GST), and (3) lipoxins by lipoxygenases. (c) The epoxygenase pathway forms epoxyeicosatrienoic acid (EET) and dihydroxyacids by cytochrome p450 epoxygenase **(fig001dfd)**.

The gene structure of *COX-2* is similar to immediate early genes such as intercellular cell adhesion molecule 1 (ICAM-1) and its expression is rapidly upregulated during inflammation and other pathological processes, including cancer. Furthermore, an AU-rich element (ARE)-containing 3'-untranslated region of COX-2 mRNA appears to be important in the normal

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regulation of rapid mRNA decay, thereby keeping COX-2 expression tightly regulated (Ref. 32). In colon cancer cells overexpressing COX-2, COX-2 mRNA was found to be turned over more slowly than normal as a consequence of defective recognition of AREs within the COX-2 mRNA. This stabilisation of COX-2 mRNA appears to be a major mechanism for constitutive overexpression of COX-2 in colon cancer (Ref. 32).

Protein sequence

At the amino acid level, COX-1 and COX-2 are 60% homologous and are composed of three main domains: a membrane-binding domain, an epidermal growth factor (EGF)-like domain and an enzymatic domain (Ref. 33). There are major differences in the N-terminal region, where COX-2 has a shorter signal peptide than COX-1, and in the membrane-binding C-terminal region, where COX-2 has an 18 amino acid insert (Ref. 33). The amino acid residues required for the catalytic activity are conserved between COX-1 and COX-2. Both enzymes are glycosylated, with three conserved *N*-glycosylation sites. An additional glycosylation site is located in the C-terminal end of COX-2. Both enzymes have similar (although not identical) K_m and V_{max} values for arachidonic acid and have similar reaction mechanisms (Ref. 34). There are subtle differences in the substrate pocket, which have been exploited to generate isoform-specific inhibitors. Thus, a valine to isoleucine substitution at position 509 in COX-1 renders the enzyme sensitive to COX-2 inhibitors (Ref. 35), by providing access to a side-channel not normally available in COX-1. The domains of the COX-1 and COX-2 proteins are summarised in Figure 2.

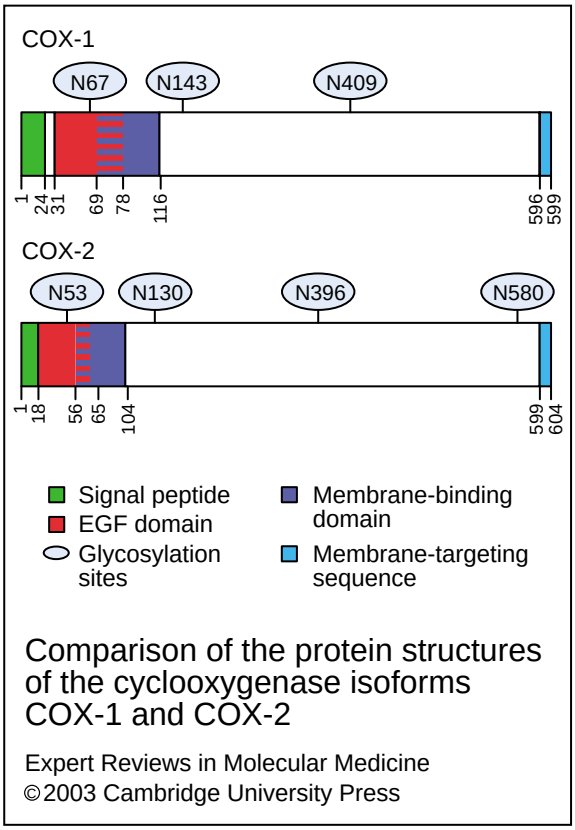

Figure 2. Comparison of the protein structures of the cyclooxygenase isoforms COX-1 and COX-2. The diagram illustrates the conserved regions between the two proteins: the signal peptide, epidermal growth factor (EGF) domain, membrane-binding domain and membrane-targeting sequence. The glycosylation sites are also shown; note the additional glycosylation site in the COX-2 protein. COX-1 and COX-2 are 60% homologous at the amino acid level, and the major differences occur at the N-terminal region, where COX-2 has a shorter signal peptide than COX-1, and in the membrane-binding C-terminal region, where COX-2 has an 18 amino acid insert. For further information on the protein structures of COX-1 and COX-2, see Refs 33, 34 and 35 **(fig002dfd)**.

Subcellular localisation

Despite their biochemical similarities, the COX-1 and COX-2 enzymes represent two independent prostaglandin biosynthetic systems and might couple to distinct pools of arachidonic acid. Both COX-1 and COX-2 are found on the luminal surface of the endoplasmic reticulum (ER) and nuclear membrane (Ref. 36). However, immunocytofluorescence studies show that COX-2 is more concentrated in the nuclear envelope than in the ER, whereas COX-1 is equally distributed in both. This difference in subcellular localisation could serve to separate the activities of COX-1 and COX-2 within cells. Consequently, COX-2, especially the subset localised to the nuclear envelope, might be a major source of prostaglandins involved in a peroxisome proliferator activated receptor (PPAR)-mediated nuclear signalling system (Ref. 37), which could explain the relationship between expression of COX-2 and cell differentiation and replication. There is also evidence that COX-2 might localise to specific regions of the cell membrane, as it has been reported to complex with caveolin (a cell membrane protein) following induction in human foreskin fibroblasts (Ref. 38).

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Prostaglandin generation in vascular disease

TXA_2 promotes platelet activation

TXA_2 is the main metabolite of arachidonic acid in platelets in normal subjects (Ref. 4), but can also be formed by nucleated cells such as monocytes (Table 1). Initial studies showed enhanced TXA_2 biosynthesis in platelets during the development of atherosclerosis in rabbits (Ref. 39). Later, it was shown that TXA_2 synthesis, as reflected by plasma TXB_2 concentration in the coronary sinus, is increased in unstable angina (Ref. 40). Furthermore, increased urinary 11-dehydro-TXB_2 and 2,3-dinor-TXB_2 is increased in patients with unstable angina and atherosclerosis (Refs 41, 42).

TXA_2 synthesis occurs as a result of platelet activation and hyperactivity, and is associated with increased plasma low-density lipoprotein (LDL) cholesterol in vascular disease (Refs 43, 44). Activated platelets release mitogenic factors such as PDGF and EGF, which promote the development of atherosclerosis by stimulating the proliferation and migration of vascular smooth muscle cells (VSMCs), leading to atherosclerotic plaque formation. The degree to which TXA_2 participates in this process is unclear. However, it is known that TXA_2 promotes platelet activation and has a direct effect on VSMCs, promoting mitogenesis through a distinct TXA_2/PGH_2 receptor (Refs 45, 46). Furthermore, TXA_2 induces phospholipase C activation, leading to the production of inositol trisphosphate (IP_3), which results in the mobilisation of intracellular Ca^{2+} in VSMCs. This leads to the induction and proliferation of VSMCs and hence contributes to plaque development. Antagonism of the TXA_2/PGH_2 receptor has been shown to retard plaque formation in hypercholesterolaemic rabbits (Ref. 47), prevent arterial thrombosis in rats (Ref. 48) and decrease atherosclerosis in the apoE$^{-/-}$ mouse, which lacks a functional gene encoding apolipoprotein E and has elevated levels of plasma LDL cholesterol (hypercholesterolaemia) (Ref. 49).

Previous studies with low-dose aspirin pointed to platelet COX-1 as the major source of the increased TXA_2 biosynthesis in atherosclerosis (Ref. 50). Aspirin inhibits platelet-mediated thrombosis at sites of vascular injury, suggesting that TXA_2 plays a role in platelet activation in vivo. However, aspirin does not reduce narrowing of a coronary artery (restenosis) following the reduction of a previous narrowing by angioplasty (Ref. 51) or removal by carotid endarterectomy (Ref. 52), and there is no evidence that aspirin influences the development and progression of atherosclerosis. Despite the suppression of platelet COX-1, incomplete suppression of TXA_2 metabolite excretion has been detected in some patients with unstable angina (Refs 42, 53). Karim and colleagues have demonstrated metabolism of endothelial-derived endoperoxides by platelet thromboxane synthase, providing a mechanism for the continued generation of thromboxane in patients with unstable angina treated with aspirin (Ref. 54). In one study, the persistent TXA_2 formation in patients with unstable angina treated with aspirin was abolished by the addition of a nonselective COX inhibitor, which together

Table 1. Summary of the biological effects of PGI_2 and TXA_2 (tab001dfd)

Eicosanoid	Source[a]	Effects
PGI_2	Endothelial cells Vascular smooth muscle cells	Antithrombotic and anti-inflammatory Induces vasodilation Inhibits platelet aggregation Inhibits platelet mitogen release Suppresses smooth muscle cell proliferation Inhibits leukocyte adhesion
TXA_2	Platelets Monocytes	Platelet activator Induces vasoconstriction Promotes mitogenesis Stimulates smooth muscle cell proliferation

[a] PGI_2 is mainly derived from the action of COX-2; TXA_2 is mainly derived from the action of COX-1.
Abbreviations: COX, cyclooxygenase; PGI_2, prostacyclin; TXA_2, thromboxane A_2.

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with other studies suggests an extra-platelet source for the increased TXA_2 (Refs 53, 55). This suggests that inhibition of COX-1 in platelets is insufficient to suppress the TXA_2 generated in atherosclerosis – possibly as other sources of TXA_2 are responsible for the enhanced TXA_2 formation seen in this disease. Current clinical studies with the TXA_2 receptor antagonist S18886 (Ref. 49) will go some way towards defining the role of TXA_2 in atherosclerosis.

PGI_2 is antithrombotic and anti-inflammatory

PGI_2 is generated by large-vessel endothelium and VSMCs (Table 1). Although some reports show a deficiency of PGI_2 in atherosclerosis (Ref. 56), most evidence shows that PGI_2 is increased in vascular disease (Refs 57, 58, 59). PGI_2 inhibits the release of mitogens such as PDGF and EGF from platelets, endothelial cells and macrophages and thus, when synthesised by endothelial cells, will suppress VSMC proliferation in plaques (Ref. 60). It also prevents the accumulation of cholesterol esters in macrophages and therefore suppresses foam cell formation (Ref. 61). PGI_2 inhibits leukocyte adhesion and activation, platelet aggregation and VSMC migration.

PGI_2 and stable analogues of the prostaglandin reduce DNA synthesis in cultured rabbit aortic smooth muscle cells and inhibit proliferation of smooth muscle cells from atherosclerotic plaque (Ref. 62). Overexpression of COX-1 by virus-mediated gene transfer increases the synthesis of PGI_2 and protects against thrombus formation (Ref. 63). Moreover, disruption of the PGI_2 receptor in mice has demonstrated an antithrombotic as well as anti-inflammatory role for PGI_2 (Ref. 64). However, COX-1 is not the major source of PGI_2 in healthy individuals. By contrast, recent studies measuring 2,3-dinor-6-keto-$PGF_{1\alpha}$ during administration of a selective COX-2 inhibitor suggest that COX-2 is the major source of PGI_2 in normal subjects (Refs 65, 66). An important question that concerns COX-2 inhibitors is whether the selective reduction of PGI_2 increases the risk of thrombosis in the face of unopposed TXA_2 generation. However, it is interesting to note that no further increase in TXA_2 generation (a marker of platelet activity) occurred in patients with vascular disease who had been administered the selective COX-2 inhibitor nimesulide, despite marked suppression of PGI_2 generation (Ref. 67).

COX expression in normal vasculature and in vascular disease

COX-1

Both endothelial cells and VSMCs express COX (Table 2), although endothelial cells contain up to 20-times more COX than do VSMCs. In large vessels, PGI_2 is formed predominantly in the endothelial layer by COX-1 (Ref. 65) and COX-2, whereas the underlying VSMCs generate considerably less prostaglandins. In small vessels, the principal product is PGE_2. Although COX-1 has traditionally been considered a constitutively expressed isoform it can also be induced in vascular cells. For example, shear stress induces COX-1 in human umbilical vein endothelial cells (HUVECs) (Ref. 68). Furthermore, vascular endothelial growth factor (VEGF) induces COX-1 expression in both bovine endothelial cells and HUVECs (Ref. 69). The induction of COX-1 is not limited to endothelial cells, as induction of this isoform in a promonocytic cell line has also been reported (Ref. 70).

In addition to COX-1 being expressed in endothelial cells and VSMCs in normal tissue, it is also expressed in atherosclerotic plaques (Table 2), predominantly in VSMCs underlying the plaque as well as in adjacent sections with normal morphology (Ref. 71). In a model of vascular injury characterised by VSMC proliferation without macrophage infiltration, there is increased COX-1 in VSMCs of the neointima (the layer of proliferating VSMCs that develops following disruption of the endothelium). This suggests that expression of COX-1 might be induced in vascular cells in vivo (Ref. 55).

COX-2

COX-2 is the predominant source of PGI_2 in humans, based on studies of metabolite excretion (Ref. 65), but the source of the COX-2 is unknown. Ex vivo studies show no evidence that COX-2 is expressed in normal blood vessels (Ref. 67). By contrast, COX-2 is expressed in atherosclerotic plaque ex vivo, which is not surprising given the role of cytokines and growth factors in its pathogenesis. The increase in COX-2 expression is evident in endothelial cells, VSMCs and macrophages (Refs 71, 72, 73, 74, 75) (Table 2). COX-2 is also expressed in VSMCs of the neointima following balloon angioplasty (Ref. 55). The increased COX-2 expression in monocytes and the subsequent generation of PGE_2 results in

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Table 2. Summary of COX-1 and COX-2 expression in cell types in normal and diseased vasculature (tab002dfd)

COX	Constitutive expression	Expression induced in atherosclerosis	Comments
COX-1	Circulating platelets Endothelial cells	Macrophage-derived foam cells Vascular smooth muscle cells Arterial thrombus	Constitutive COX-1 expression in platelets generates TXA_2; increased COX-1 expression and TXA_2 production in advancing atherosclerosis promotes thrombosis
COX-2	–	Endothelial cells Macrophages Vascular smooth muscle cells	Increased COX-2 expression in atherosclerosis increases PGI_2 generation

Abbreviations: COX, cyclooxygenase; PGI_2, prostacyclin; TXA_2, thromboxane A_2.

induction of matrix metalloproteinases (MMPs) 2 and 9, and therefore might be involved both in the development of lesions and in plaque rupture (Ref. 75). However, PGI_2, the main product in vascular cells, inhibits MMPs, suggesting that there might be a negative-feedback pathway (Ref. 76). It is worth noting that morphologically stable plaques express less COX-1, COX-2 and MMPs in comparison with unstable lesions, perhaps reflecting the greater abundance of macrophages in the latter.

COX-2 expression in macrophages and VSMCs generates eicosanoids that might be expected to have proinflammatory effects such as increased vascular permeability, chemotaxis and cell proliferation (Ref. 77). COX-2 limits cell death (a feature of atherosclerotic plaques) in several tissues, including cardiomyocytes (Ref. 25) and epithelial cancers (Ref. 78) and so indirectly could promote VSMC growth. Prostaglandins might also induce mitogenesis directly. TXA_2, $PGF_{2\alpha}$ and the isoprostane 8-iso-$PGF_{2\alpha}$ (a free-radical-derived product of arachidonic acid) induce proliferation of VSMCs (Refs 79, 80, 81, 82). COX-2-derived prostaglandins mediate these processes through activation of G-protein-coupled transmembrane receptors and therefore might contribute to lesion development. Alternatively, prostaglandins might limit lesion development. Overexpression of COX-2 or prostacyclin synthase suppresses the development of vascular lesions and the growth of VSMCs (Refs 83, 84). These effects might be mediated through PPARs (Refs 85, 86), which are a series of nuclear membrane receptors that heterodimerise with other transcription factors. Activation of PPAR-γ in macrophages and foam cells inhibits the expression of activated genes such as inducible nitric oxide synthase, MMP-9 and scavenger receptor A. PPAR-γ might also affect monocyte recruitment in atherosclerotic lesions through the regulation of vascular cell adhesion molecule 1 (VCAM-1) and ICAM-1 in vascular endothelial cells. Furthermore, a partial PPAR-γ agonist has been shown to reduce atherosclerosis in $LDLR^{-/-}$ mice (Ref. 87). As COX-2 preferentially localises in the perinuclear region, its products could have more access to nuclear receptors. Indeed, PPAR-γ, which is expressed in macrophage-derived foam cells (Ref. 88), has been reported to regulate COX-2 through a negative-feedback loop (Ref. 89). The natural ligand for PPAR-γ in this setting is unknown. However, the COX products PGJ_2 and its metabolite 15-deoxy-PGJ_2 are PPAR-γ agonists and have been shown to regulate the expression of genes in VSMCs, including those involved in cell growth and/or apoptosis (Ref. 90).

COX and the cardiovascular system

Selective COX-2 inhibitors

NSAIDs are widely used to treat arthritis, dental pain and other inflammatory conditions. However, their long term use has been limited by GI side effects and renal problems. NSAIDs inhibit both COX-1 and COX-2. The inhibition of COX-2 has been implicated in treating inflammation whereas the inhibition of COX-1 has been related to the adverse effects.

Three classes of cyclooxygenase inhibtiors exist. Aspirin is the only NSAID that covalently modifies COX. Aspirin inhibits both COX-1 and COX-2 by acetylation of Ser530 and Ser516,

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respectively, in the substrate-binding site. This excludes the access of arachidonic acid to Tyr385 by steric hindrance (Ref. 91). However, aspirin is 10–100-times more potent against COX-1 than COX-2 as the acetyl-serine side chain can rotate in the slightly larger site of COX-2, allowing limited access of substrate to the active site (Ref. 92). Various experimental models have been used to test the selectivity of NSAIDs in vitro, including purified enzymes, intact cell systems and cells transfected with recombinant enzymes. The selectivity of the compound is evaluated by calculating a ratio of the IC50 values for COX-2 and COX-1. However, depending on the model used, the absolute IC50 value and values for the IC50 ratio of COX-2:COX-1 vary, although the order of selectivity stays constant from one model to another. Naproxen and diclofenac are equipotent in inhibiting COX-1 and COX-2, whereas indomethacin, piroxicam, sulindac and tolmetin are more active against COX-1 than COX-2 (Ref. 93). Development of coxibs, the selective COX-2 inhibitors, is a response to the poor therapeutic profile of traditional NSAIDs. While the important residues for catalysis are conserved between COX-1 and COX-2, the substitution of a valine at position 509 in the cyclooxygenase site of COX-2 for an isoleucine in COX-1 has allowed for the generation of COX-2 selective inhibitors. It has been shown by site-directed mutagenesis that V509I COX-2 behaves like COX-1, being unresponsive to inhibition by COX-2 selective inhibitors (Ref. 94).

All the selective COX-2 inhibitors described to date are reversible inhibitors of COX-2 and relatively poor inhibitors of COX-1, as a result of the one amino acid difference within the hydrophobic cyclooxygenase channel. The coxibs represent a new class of anti-inflammatory drugs that are better tolerated than traditional NSAIDs but equally efficacious. The first selective COX-2 inhibitors to be developed were celocoxib (Ref. 95) and rofecoxib (Ref. 96). Subsequently, other coxibs such as valdecoxib (Ref. 97) and etoricoxib (Ref. 98) have been developed. To date, the efficacy of coxibs in the treatment of arthritis, the relief of acute pain and the lower incidence of GI side effects have been documented in clinical studies (Refs 11, 99, 100).

The development of vascular disease

Much attention has focused on the relative contributions of COX-1 and COX-2 to the development of atherosclerosis in vivo. These studies have been facilitated by the development of coxibs (Ref. 96).

Administration of coxibs provides a means of studying the contribution of COX-2 both to prostaglandin formation in vascular disease and to the development of vascular lesions. In a rat model of balloon angioplasty of the carotid artery, both COX-1 and COX-2 expression were found to be increased in VSMCs of the neointima generated (Ref. 55). This was associated with an increase in urinary TXA_2 and PGE_2 generation that was in part suppressed by the coxib SC236. However, this coxib had no effect on neointima formation. By contrast, administration of either the selective COX-1 inhibitor SC-560 or the nonselective NSAID indomethacin suppressed the intimal hyperplasia. Thus, whereas both isoforms were responsible for the increase in prostaglandin generation in the animals following vascular injury, only COX-1 appeared to play a role in the development of restenosis. COX-1 has also been implicated in a model of atherosclerosis (Ref. 101).

COX-1 expressed in vascular lesions might play a role in lesion development, have a protective effect or regulate platelet deposition. For example, overexpression of COX-1 in vascular tissue using an adenoviral vector protects against angioplasty-induced arterial thrombosis in a pig model (Ref. 102). Alternatively, the effects of SC-560 might be due to the suppression of platelet and/or vascular TXA_2, a potent platelet activator and mitogen for VSMCs. This is supported by recent findings showing that continuous administration of aspirin retards the development of atherosclerosis in $apoE^{-/-}$ mice (Ref. 103).

Other studies suggest a role for COX-2 in atherosclerosis. Pratico and colleagues reported a 30% reduction in atherosclerosis by nimesulide in the $apoE^{-/-}$ murine model. Burleigh et al. also demonstrated that administration of the coxib rofecoxib reduced atherosclerosis in a mouse model deficient in the LDL receptor (LDLR) (Ref. 104). In this study, repopulation of the $COX\text{-}2^{+/+}LDLR^{-/-}$ mouse with monocytes from a COX-2-knockout mouse also inhibited atherosclerosis, implicating monocyte-expressed COX-2 alone in the development of atherosclerosis.

In human atherosclerosis and in experimental mouse models, generation of both TXA_2 and PGI_2 is increased (Ref. 105). TXA_2 is largely derived

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from COX-1, at least on the basis of inhibitor studies. Thus, low-dose aspirin suppresses TXA_2 formation whereas selective COX-2 inhibition has little effect. By contrast, both COX-1 and COX-2 are responsible for the increase in PGI_2 generation (Ref. 67). Indeed, expression of both isoforms is increased in human atherosclerotic lesions, with several cell types expressing COX-2, including VSMCs and monocytes (Refs 106, 107) (Table 2). Whether specific inhibition of either isoform influences the development of atherosclerosis is unknown. There is also much evidence from clinical trials that aspirin protects against stroke and myocardial infarction (Ref. 108). Although aspirin has been reported in a small subset of individuals to retard carotid plaque progression (Ref. 109), there is no evidence that NSAIDs influence lesion formation (Ref. 110). Indeed, possibly as they are reversible inhibitors of COX-1 in contrast to aspirin, NSAIDs appear to have no effect in vascular disease.

Angiogenesis

Angiogenesis is an essential process in tumour growth. Both COX isoforms appear to modulate angiogenesis. Neovessel formation requires the presence of COX-2, which mediates the synthesis of angiogenic factors (Refs 111, 112). Indeed, selective inhibition of COX-2 in neovascular tumours that support human cancers suggests an antitumour role for COX-2 inhibition by inhibition of cell proliferation and angiogenesis (Refs 113, 114, 115). Furthermore, TXA_2 might prevent and reverse angiogenesis (Ref. 5) via endothelial apoptosis during conditions such as myocardial infarction or myocarditis, in which TXA_2 is formed and released from platelets or macrophages. Therefore, it is reasonable to hypothesise that COX-2 might contribute to the formation of new blood vessels in the development of atherosclerosis, thereby allowing the plaque to expand. Indeed, the COX-2-derived production of PGE_2 and PGI_2 can directly stimulate endothelial cell migration and growth-factor-induced angiogenesis (Refs 116, 117). However, the microvascular endothelium expresses both COX-1 and COX-2, raising the possibility that the presence of both isoforms contributes to the formation of new blood vessels within the plaque. Indeed, previous work has shown that VEGF induces COX-1-dependent proliferation of endothelial cells (Ref. 69). In addition, Tsujii et al. have demonstrated that COX-1 activity in endothelial cells modulates angiogenesis (Ref. 118). Conversely, other authors have shown that COX-2-derived prostaglandins regulate angiogensis in endothelial cells (Ref. 119) and in animal models of inflammation (Ref. 120). Therefore, it seems reasonable to suggest that both COX isoforms are involved in the angiogenic response.

In atherosclerosis, the development of angiogenesis could have both beneficial and deleterious effects. On the one hand, increased angiogenesis might play a role in the healing of ischaemic tissues (Ref. 121); on the other hand, angiogenesis in an atherosclerotic lesion might also result in plaque expansion and vulnerability (Refs 122, 123, 124, 125). It has recently been demonstrated in vitro that both nonselective (indomethacin) and COX-2-selective (NS-398) NSAIDs inhibit hypoxia-induced angiogenesis in gastric microvascular endothelial cells (Ref. 126), suggesting a role for COX-2 in angiogenesis in endothelial cells .

Selective COX-2 inhibition and cardiovascular disease

Selective inhibition of COX-2 with coxibs has been used to treat arthritis for several years (Ref. 13). As atherosclerosis is an inflammatory process in which COX-2 expression is induced and subsequent prostaglandin generation is increased, selective inhibition of COX-2 might be expected to influence atherosclerosis. The transformation of macrophages to foam cells in response to acetylated LDL is associated with decreased PGI_2 and PGE_2 generation (Ref. 127). PGE_2, through the EP4 receptor and cAMP elevation, regulates the stability of COX-2 mRNA in a negative-feedback loop. Consequently, it has been proposed that a reduction in PGE_2 generation during macrophage and foam cell formation might downregulate COX-2 expression and could attenuate the inflammatory response (Ref. 128). However, based on data from animal models and results of clinical trials investigating GI safety of coxibs (see below), it is unlikely that the effect of selective COX-2 inhibition on the development of atherosclerosis will be examined in a human population.

Recently, two large trials addressing the efficacy of coxibs and the associated risk of GI complications have reported on the frequency of cardiovascular events. In the VIGOR study (Vioxx Gastrointestinal Outcomes Research Study) (Ref. 13), the occurrence of GI toxicity with

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the coxib rofecoxib (50 mg once daily) or the NSAID naproxen (500 mg twice daily) was compared in 8076 patients with rheumatoid arthritis. In this study, aspirin use was not permitted. The incidence of GI perforation, haemorrhage or symptomatic peptic ulcer was significantly less in the rofecoxib group compared with the naproxen group. The CLASS trial (Celocoxib Long-Term Arthritis Safety Study) (Ref. 129) compared the coxib celocoxib (400 mg twice daily) with the NSAID diclofenac (75 mg twice daily) and celocoxib with the NSAID ibuprofen (800 mg three times daily). Aspirin use was permitted in all CLASS trial patients. There was no significant difference in the incidence of primary endpoint of ulcer or GI bleeding in either NSAID group.

In the VIGOR trial, the rates of nonfatal myocardial infarction and death from any vascular event was higher in the rofecoxib group than in the naproxen group (0.8% versus 0.4%, $P < 0.05$). It is important to note that 4% of the patients in the VIGOR trial met the Food and Drug Administration (FDA) criteria for the use of aspirin therapy. Conversely, in the CLASS trial, there were no differences in cardiovascular events between the celocoxib, diclofenac or ibuprofen groups. However, in this trial, 21% of the patients received aspirin therapy during the study. As a result, there have been suggestions that long-term administration of coxibs might increase the risk of cardiovascular events (Refs 14, 130). However, there are important issues that need to be addressed that might in part explain the discrepancies observed between the two studies.

It has been suggested that the results of the VIGOR study could be explained by an antithrombotic effect of naproxen. Naproxen has significant antiplatelet effects with a mean platelet aggregation inhibition in the region of 90% (Ref. 131), which is comparable with aspirin (92%). Therefore, naproxen, but not ibuprofen (platelet aggregation 80%) or diclofenac (platelet aggregation of 40%) as used in the CLASS trial, might be expected to achieve a strong inhibition of platelet aggregation. However, this feature of naproxen has been disputed (Ref. 132) and indeed case–control studies in large populations suggest that naproxen has no effect on the risk of events in patients with coronary artery disease (Ref. 133).

An alternative explanation that is consistent with the biology of prostaglandins in the cardiovascular system is that COX-2 inhibitors, by virtue of their inhibition of PGI_2 without a corresponding reduction in platelet TXA_2, are prothrombotic (Fig. 3). Cheng and colleagues, in studies of prostacyclin-receptor-knockout (IPKO) and thromboxane-receptor-knockout (TPKO) mice, showed that endogenous PGI_2 modulated the cardiovascular effects of TXA_2. Thus, vascular proliferation and platelet activation in response to injury were enhanced in IPKO but decreased in TPKO animals. Furthermore, the augmented response to vascular injury in the IPKO animals was abolished when both the prostacyclin receptor and thromboxane receptors were deleted (Ref. 134). These findings might in part explain the cardiovascular effects of coxibs in the VIGOR study. In normal subjects (Ref. 65) and in atherosclerosis (Ref. 67), a substantial proportion of endogenous PGI_2 is generated by COX-2, so that selective COX-2 inhibitors suppress PGI_2 formation by as much as 80% with little effect on TXA_2. However, it should be emphasised that targeted gene deletion of the prostacyclin receptor might greatly exceed the effect achieved by COX-2 inhibitors, where there is continued generation of PGI_2. Moreover, the precise source of the PGI_2 inhibited by COX-2 inhibitors is unknown, although the vasculature is a major source of this prostaglandin.

A further explanation for the differences between the two trials is differences in the cardiovascular risk in the populations studied. Patients with rheumatoid arthritis (Ref. 135) such as those in the VIGOR trial are reported to have an increased rate of cardiovascular events, although this is disputed. This has not been observed in patients with osetoarthritis (Ref. 136), who constituted the majority of patients in the CLASS trial. However, it is important to note that the incidence of myocardial infarction was very similar in the two populations treated with the COX-2 inhibitors (0.8% for celocoxib; 0.74% for rofecoxib).

Conclusions

Selective COX-2 inhibitors have been developed as a new class of inhibitors used in the treatment of such conditions as arthritis and cancer. Their advantage of not causing gastric ulceration as is observed with the other NSAIDs is widely recognised. However, recently their long-term use has been challenged by reports that selective inhibition of COX-2 is associated with increased cardiovascular events such as

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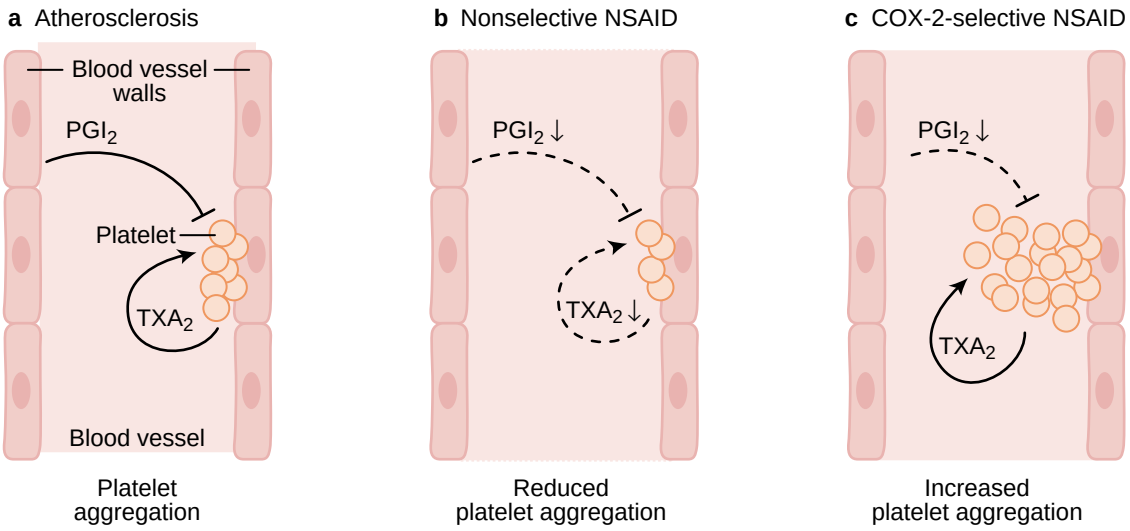

Figure 3. Possible mechanisms underlying the effect of selective COX-2 inhibition in atherosclerosis. (a) In atherosclerosis, prostacyclin (PGI_2) generation inhibits thromboxane A_2 (TXA_2)-induced platelet activation and aggregation. (PGI_2 is prduced by endothelial cells; TXA_2 is produced by platelets.) (b) Administration of a nonselective, nonsteroidal anti-inflammatory drug (NSAID) decreases generation of both TXA_2 and PGI_2 (dashed lines), leading to reduced platelet aggregation. (c) Selective inhibition of COX-2 decreases PGI_2 without a concomitant inhibition of TXA_2, and hence increases platelet aggregation **(fig003dfd)**.

myocardial infarction. It is clear that expression of both COX-1 and COX-2 increases in vascular disease, resulting in enhanced prostaglandin generation. COX-2 is expressed in the monocytes/macrophages and proliferating VSMCs that typify atherosclerotic lesions, in addition to endothelium. Patients with extensive disease have enhanced formation of TXA_2, a potent platelet activator and vasoconstrictor, largely derived from COX-1 in platelets. Formation of PGI_2, a potent platelet inhibitor and vasodilator, is also increased largely through COX-2. Thus, in atherosclerosis, COX-2 inhibition preferentially suppresses PGI_2 generation and spares TXA_2, as it also does in normal individuals. Theoretically, PGI_2 might limit the extent of platelet adhesion and activation at sites of vascular disease. Given that PGI_2 regulates the response to TXA_2, and COX-2 inhibition selectivity suppresses PGI_2, it follows that COX-2 inhibitors might enhance platelet activity (Fig. 3). This provides a plausible explanation for the increased risk of myocardial infarction reported with the COX-2 inhibitor rofecoxib. There are data demonstrating that COX-2 expressed in monocytes/macrophages contributes to the development of atherosclerosis in murine models and to the expression of proteins such as MMPs that contribute to plaque instability. In that case, inhibition of COX-2 would be expected to limit the extent of atherosclerosis. However, more evidence suggests a role for COX-1-mediated prostaglandin generation in the development of atherosclerosis. Selective inhibition of COX-1 reduces lesion formation, possibly reflecting an antiplatelet effect; this is further supported by studies showing that nonselective COX inhibitors, such as aspirin and indomethacin, retard the development of atherosclerosis animal models to a greater extent than do selective COX-2 inhibitors.

However, the specific role of COX-1 and COX-2 in vascular regulation is still unknown and knowledge of this is imperative to the future use of coxibs. More information is required on the pharmacology and clinical use of selective COX-2 inhibitors – in particular, their combination

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with antiplatelet drugs. In the interim, it seems reasonable to suggest that in patients with a history of a cardiovascular event, coxibs should be used in combination with low-dose aspirin. Aside from potentially increasing the rate of myocardial infarction, selective inhibition of COX-2 might have relevance to other aspects of cardiovascular biology such as hypertension, atherogenesis and cardiac function.

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Textbooks

Zipes, D.P. and Libby, P., eds (2001) Heart Disease: A Textbook of Cardiovascular Medicine, WB Saunders Company

Websites

The JRAWorld and Arthritis Insight website provides useful information on the mechanism of action and effects of NSAIDs:

http://jraworld.arthritisinsight.com/medical/meds/nsaidlinks.html

The British Heart Foundation site has many useful articles for patients:

http://www.bhf.org.uk

Other useful websites:

http://www.jr2.ox.ac.uk/bandolier/band52/b52-2.html
http://www.nsaid.net/
http://www.coreynahman.com/arthritis_drugs_database_nsaids.html
http://www.psychiatry.wustl.edu/Resources/LiteratureList/2001/September/Fitzgerald.pdf

Features associated with this article

Figures

Figure 1. The three major pathways involved in arachidonic acid metabolism (fig001dfd).
Figure 2. Comparison of the protein structures of the cyclooxygenase isoforms COX-1 and COX-2 (fig002dfd).
Figure 3. Possible mechanisms underlying the effect of selective COX-2 inhibition in atherosclerosis (fig003dfd).

Tables

Table 1. Summary of the biological effects of PGI_2 and TXA_2 (tab001dfd).
Table 2. Summary of COX-1 and COX-2 expression in cell types in normal and diseased vasculature (tab002dfd).

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