Cyclooxygenase-2 Expression and Inhibition in Atherothrombosis

Francesco Cipollone, Bianca Rocca, Carlo Patrono

Abstract—Arachidonic acid metabolism plays an important role in acute ischemic syndromes affecting the coronary or cerebrovascular territory, as reflected by biochemical measurements of eicosanoid biosynthesis and the results of inhibitor trials in these settings. Two cyclooxygenase (COX)-isozymes have been characterized, COX-1 and COX-2, that differ in terms of regulatory mechanisms of expression, tissue distribution, substrate specificity, preferential coupling to upstream and downstream enzymes, and susceptibility to inhibition by the extremely heterogeneous class of COX-inhibitors. Although the role of platelet COX-1 in acute coronary syndromes and ischemic stroke is firmly established through ≈20 years of thromboxane metabolite measurements and aspirin trials, the role of COX-2 expression and inhibition in atherothrombosis is substantially uncertain, because the enzyme was first characterized in 1991 and selective COX-2 inhibitors became commercially available only in 1998. In this review, we discuss the pattern of expression of COX-2 in the cellular players of atherothrombosis, its role as a determinant of plaque “vulnerability,” and the clinical consequences of COX-2 inhibition. Recent studies from our group suggest that variable expression of upstream and downstream enzymes in the prostanoid biosynthetic cascade may represent important determinants of the functional consequences of COX-2 expression and inhibition in different clinical settings. (Arterioscler Thromb Vasc Biol. 2004;24:246-255.)

Key Words: atherothrombosis ■ COX-2 ■ COX-inhibitors ■ platelets ■ inflammatory cells

Arachidonic acid metabolism plays an important role in acute ischemic syndromes affecting the coronary or cerebrovascular territory, as reflected by biochemical measurements of eicosanoid biosynthesis and the results of inhibitor trials in these settings. In particular, the clinical efficacy of low-dose aspirin in reducing the short-term complications of acute myocardial infarction and acute ischemic stroke, as well as in preventing vascular recurrences, has focused attention on the cyclooxygenase (COX) pathway of arachidonic acid metabolism and its bioactive products. These include D, E, F, and I prostaglandins (PGs) and thromboxane (TX) A2, collectively termed prostanoids (Figure 1). Prostanoid biosynthesis involves 3 sequential enzymatic steps: (1) agonist-induced phospholipase (PL) activation to release arachidonic acid from membrane phospholipid pools; (2) COX-catalyzed oxygenation of the free fatty acid to generate the cyclic endoperoxide, PGH2; and (3) enzymatic rearrangement of PGH2 structure to yield one of several bioactive derivatives (Figure 1). Although the first 2 steps are shared by virtually all human cell types, the expression of downstream prostanoid synthases displays considerable cell type specificity. An additional layer of complexity in prostanoid biosynthesis is represented by the existence of different lipid precursors (eg, 2-arachidonylglycerol and anandamide in addition to C:20-fatty acids), as well as by the existence of different isoforms of PL, COX, and prostanoid synthases.

In particular, 2 COX-isozymes have been characterized, COX-1 and COX-2, that differ in terms of regulatory mechanisms of expression, tissue distribution, substrate specificity, preferential coupling to upstream and downstream enzymes, and susceptibility to inhibition by the extremely heterogeneous class of COX inhibitors. Although the role of platelet COX-1 in acute coronary syndromes and ischemic stroke is firmly established through ≈20 years of TX metabolite (TXM) measurements and aspirin trials,1,2 the role of COX-2 expression and inhibition in atherothrombosis is substantially uncertain, because the enzyme was first characterized in 1991 and selective COX-2 inhibitors became commercially available only in 1998.3

The aim of this article is to review the pattern of expression of COX-2 in the cellular players of atherothrombosis, its role as a determinant of plaque “vulnerability,” and the clinical consequences of COX-2 inhibition. Although focusing on COX-2, we will also develop the theme that variable expression of upstream and downstream enzymes in the prostanoid biosynthetic cascade may represent important determinants of the functional consequences of COX-2 expression and inhibition in different clinical settings.
Expression and Regulation of COX-2 in Circulating Blood Elements and Early Atherogenesis

All circulating blood elements participate in atherogenesis, including platelets, monocytes, neutrophils, and lymphocytes. Despite the widely recognized role of COX-2 in human inflammatory disorders, the net effect of COX-2 expression in the different phases of atherogenesis remains controversial.

Adhesion of circulating leukocytes, especially monocytes, to activated endothelial cells appears as a critical early event observed in initial atherosclerotic lesions, allowing subsequent migration of bloodborne cells into the arterial intima. COX-2 has been detected in the fatty streaks of both humans and mice. Monocyte adhesion to activated endothelial COX-2 has been detected in the fatty streaks of both humans and mice.10,11 Monocyte adhesion to activated endothelial COX-2 has been detected in the fatty streaks of both humans and mice.

Figure 1. Production and actions of prostanoids originating via the COX-2 pathway. Arachidonic acid, a 20-carbon polyunsaturated fatty acid, is released from the sn2 position in membrane phospholipids by PLA2 (cPLA2 and sPLA2), which is activated by diverse stimuli. Arachidonic acid is converted by cyclooxygenase synthases, which have both COX and hydroperoxidase activity, to the unstable intermediate PGH2. PGH synthases are catalytically active in multiple prostanoids. The conversion of PGH2 to PGE2 is catalyzed by 2isoforms of the enzyme PGES (microsomal [cPGES and mPGES, respectively]). These bioactive lipids activate specific cell-membrane receptors belonging to the superfamily of G protein–coupled receptors: TP, thromboxane receptor; DP1, PGD2 receptors; EPs, PGE2 receptors; and IP, prostacyclin receptor. In addition, the endocannabinoids, 2-arachidonylglycerol (2-AG) and anandamide (data not shown), are also liberated from the cell membrane by phospholipases in a stimulus-induced manner. COX-2 can generate their oxygenation to provide PGH2 glycerol ester (PGH2-G) and PGH2-ethanolamide (data not shown). mPGES catalyzes the isomerization of PGH2-G to PGE2 glycerol ester (PGE2-G) at rates approaching those observed with PGH2. Metabolism of 2-AG into TXA2, PGD2, and PGI2 glycerol esters has also been described.

Figure 2. COX-2 expression in the different cellular players of atherothrombosis. a, COX-2 expression in monocytes during homing to the sub-endothelium. Note the positivity for COX-2 not only in monocytes/macrophages migrated into the vessel intima (arrowheads) but also in monocytes still adherent to the endothelium (arrow). b, Expression of COX-2 and PGES in platelets isolated from patients with high platelet regeneration rate owing to recent stem cell transplant. Top, Double immunostainings for the platelet-specific CD61 antigen (left) and for COX-2 (right); note that only a fraction of CD61 positive platelets express COX-2. Bottom, Double immunostainings for COX-2 (left) and the microsomal PGES-1 (right); a higher magnification of 1 platelet is shown in the insert. c, Immunohistochemistry (5X) showing staining for COX-2 in the shoulder of a vulnerable plaque. At higher magnification (63X) (insert), a prevalent COX-2 localization in perivascular macrophages is apparent. d, Confocal microscopy showing staining for COX-2 (green) on plaque-derived macrophages and microsomal PGES-1 (red); a colocalization of the 2 antigens is evident, leading to an orange color combination.

factor (NF)-κB or peroxisome proliferator-activated receptor-γ (PPAR-γ), as for ox-LDL. On the other hand, IL-4, IL-6, or IL-1 receptor antagonists, considered antiatherogenic ILs, downregulate monocytic COX-2. TNF-α has been reported to have no effect or to inhibit monocyte COX-2, depending on the length of exposure, indicating that TNF-α may play dual roles in inflammation by differential regulation of COX-2. Furthermore, prostanoids may promote chemotaxis, as suggested by a rapid release of arachidonic acid from monocytes exposed to monocyte chemotactic protein-1 (MCP-1) and by the indomethacin-induced reduction of LDL-dependent chemotaxis. The kinetics of these phenomena strongly suggests COX-2 involvement; nevertheless, experiments using selective inhibitors are lacking.

A proatherogenic role for monocyte COX-2 early in atherogenesis is suggested by studies in LDL receptor-deficient mice, in which the formation of vascular fatty streaks was reduced by the highly selective COX-2 inhibitor, rofecoxib, or by reconstituting irradiated mice with COX-2–null hematopoietic cells. Two additional studies failed to show any influence of pharmacological COX-2 inhibition on progression of atherosclerosis using LDL receptor or apolipoprotein (Apo)-E knockout mice. Analyses of aortic lesions were performed at different time points in the 3 studies: 8 weeks in the study of Burleigh et al, and 16 and 26 weeks in the study of Olesen et al and Pratico et al.
respectively. Consistently, the histology of the lesions was substantially different: 8-week-lesions resemble fatty streaks, whereas older lesions are more similar to advanced atherosclerotic plaques. These findings may imply a different biological role and relevance of COX-2 at early versus later stages of atherogenesis. At variance with these studies, showing some protection or no effect of COX-2 inhibitors during atherogenesis, an acceleration of lesion progression in Apo-E–deficient mice has been recently reported, after 3-week treatment with a highly selective COX-2 inhibitor.33

In addition to monocytes, circulating polymorphonuclear cells (PMNs) adhere to IL-stimulated endothelial cells, undergoing activation. Two important mechanisms of aspirin-insensitive formation of vasoactive eicosanoids have been characterized in PMNs, ie, the formation of leukotrienes (LTs) by 5-lipoxygenase (5-LOX), and TXA2 production by COX-2. We have recently detected enhanced biosynthesis of the potent vasoconstrictor LTC4 during the acute phase of unstable angina, and the ability of glucocorticoids to down-regulate this phenomenon.34 In addition, Mehrabian et al35 have demonstrated a critical role of 5-LOX in atherosclerosis susceptibility in mice, and we have characterized 5-LOX as an important gene contributing to atherosclerotic plaque instability in humans.36 Although LTs are the main eicosanoids from activated PMN, growing evidence indicates that COX-2 is upregulated in PMN stimulated by proatherogenic TNF-α or granulocyte-monocyte colony–stimulating factor with a parallel increase in TXA2 and PGE2 production.37–39 Interestingly, the pattern of regulation of COX-2 in PMNs versus monocytes displays distinct features,37,38 including a faster induction in PMNs, different signal transduction pathways, relative insensitivity of PMN COX-2 to glucocorticoid inhibition, lower IL-1β-dependent COX-2 induction in PMNs, and scarce or absent inhibition of PMN COX-2 by antiatherogenic ILs such as IL-4 and IL-10 compared with monocytic COX-2.39–41 Moreover, COX-2 is upregulated at early time points in circulating PMNs after injection of LPS in humans, whereas monocytic COX-2 is not affected.42 Interestingly, TXA2 seems prevalent from COX-2 activity of PMNs, whereas PGE2 is predominant from monocytic COX-2. TXA2 causes platelet activation and vasoconstriction, enhances chemoattractant MCP-1 and adhesion molecule expression on endothelial cells,43–46 and increases leukocyte adhesiveness.44,46 COX-2–dependent formation of the isoprostane 8-iso-PGF2α is derived from leukocytes may also facilitate atherogenesis, because isoprostanes enhance monocyte/endothelium adhesion.44

Thrombosis complicates established atherosclerotic lesions, and platelets are crucial contributors. COX-1 is the prevalent isoform in mature platelets, coupled with TX-synthase as the most abundant PGH2-isomerase. TXA2 plays a pivotal role in cardiovascular disorders, as demonstrated by the antithrombotic effects of low-dose aspirin, which largely reflect platelet COX-1 inhibition.48 At variance with COX-1, the presence and activity of COX-2 in platelets is more controversial. COX-2 expression in human platelets has been reported,49–51 although it does not seem to contribute to prostanooid formation during whole-blood clotting.51,52 This apparent discrepancy between platelet COX-2 expression and activity has been reconciled by the demonstration that only the youngest platelets express COX-2 derived from the parent megakaryocytes51 (Figure 2b). Thus, COX-2 is physiologically present only in a small fraction (<8%) of circulating platelets, but COX-2–expressing platelets increase substantially in conditions of high platelet regeneration.51 Human platelets can synthesize proteins such as bcl-3 and IL-1β from preformed mRNAs in an activation-dependent fashion.53,54 Whether this regulated translation applies to platelet COX-2 mRNA at sites of inflammation or thrombosis is presently unknown. PGE2 represents the main product of platelet COX-2 activity, although under high platelet turnover, a detectable amount of TXA2 is also COX-2 derived.51 The relevance of these findings to human cardiovascular diseases is currently being investigated.

Human red blood cells (RBCs) respond to picomolar concentrations of PGE2 by altering their deformability and volume,55,56 can release AA through a specific PLA2,56 and can further metabolize it via the COX pathway, primarily to PGE2 (data not shown). Even though RBCs represent the majority of circulating elements, platelets are by far more enzymatically active than are RBCs. Both COX isozymes are present in medullary RBC precursors; however, considering the long lifespan (~120 days) and lack of a nuclear apparatus for de novo protein synthesis in mature RBCs, it is unlikely that COX-1 or -2 expression may last for their whole lifespan. Indeed, only a fraction of circulating RBCs express COX-isozymes with variable intensity (data not shown). The higher prostanoid biosynthetic capacity of erythrocytes from patients with enhanced erythropoiesis is consistent with the hypothesis that COX activity is confined to newly formed cells. Preincubation with RBC facilitates platelet activation or recruitment, and this facilitatory effect is completely suppressed by the administration of a single, high dose of aspirin (500 mg), whereas a lower dosage (50 mg/d) for up to 15 days, a regimen that would completely inactivate platelet COX-1, is ineffective.57 These results are compatible with a role of erythrocyte prostanooids in promoting platelet activation and recruitment at sites of vascular injury.

Transcellular metabolism among circulating elements might be relevant in atherogenesis and in modifying the response to antithrombotic therapy. Activated platelets, which express P-selectin and CD40, can upregulate COX-2 expression in cells bearing ligands for both molecules such as leukocytes and endothelial cells.53,14,58,59 Interestingly, circulating leukocyte-platelet aggregates have been observed in unstable angina patients.50 Under these circumstances, COX-2 upregulation might provide PGH2 to platelet TX-synthase,61 thus facilitating aspirin-insensitive TXA2 biosynthesis. Microparticles released from platelets following activation have been hypothesized to carry bioactive lipids modulating multicellular interactions between endothelial cells and monocytes, including COX-2 upregulation in both cell types.62–64

Expression and Regulation of COX-2 Within the Vessel Wall and Advanced Atherogenesis

In addition to a proatherogenic role of leukocyte COX-2 in the early phases of atherogenesis, an atheroprotective role of
vascular COX-2 has been hypothesized, based on reduction of PG12 biosynthesis after coxib administration to healthy subjects.65 PG12 is considered antiatherogenic, causing vasodilation and platelet inhibition. COX-2–dependent PG12 production by endothelial cells has been reported to be modulated in vitro by laminar shear stress,66 thrombin,67 microparticles shed from activated platelets,64 oxidized cholesterol,68 CD40 engagement,58 IL-1β,69 HDL,70 and LDL.71 Therefore, endothelial COX-2 expression may represent a negative feedback mechanism triggered in part by proatherogenic/thrombotic stimuli inducing proinflammatory COX-2 in leukocytes and macrophages.

Interestingly, PG12 synthesis from human aorta samples decreases as a function of progressing atherosclerotic lesions, whereas PG12 increases in parallel.72 However, low or even undetectable levels of COX-2 have been reported in normal human arteries, and COX-2 appears predominantly expressed in endothelial cells overlying vascular lesions in the carotid, aortic, or coronary districts.10,73–75 Similar observations have been reported in normal versus atherosclerotic aortas from cholesterol-fed rabbits,76 and aortas from 8-week-old LDL-receptor–deficient mice expressed approximately one half of COX-2 mRNA-levels compared with those of older mice with well-established atherosclerotic lesions.31 This morphological evidence is not consistent with a constitutive expression of COX-2, at least in arteries, in the presence of normal flow conditions. In addition, a reduction in PG12 metabolite excretion was not associated with enhanced platelet activation, as reflected by TXA2 metabolite excretion, in atherosclerotic patients treated with COX-2 inhibitors,75 indicating that the 2 phenomena are not necessarily interdependent. The interpretation of clinical studies of selective COX-2 inhibitors is complicated by the largely unpredictable cardiovascular effects of comparator COX-1/COX-2 nonselective inhibitors (see below). Furthermore, the relative contribution of COX-1 and COX-2 to transient changes in PG12 biosynthesis that occur coincidentally with episodes of platelet activation remains to be investigated.

Within established human atherosclerotic lesions, COX-2 is largely expressed by resident macrophages77,78 (Figure 2c) and, to a lesser extent, by smooth muscle cells. However, it should be noted that many areas of atherosclerotic plaques that contain foam cells do not stain for COX-2.21,77 (Figure 2c), thus suggesting that macrophage COX-2 may be downregulated in mature foam cells. Whereas endothelial cells predominantly release PG12, macrophages synthesize an array of prostanooids, including PGE2, a proatherogenic eicosanoid when released within advanced atherosclerotic plaques. In particular, production of matrix metalloproteinase (MMP)-2 and MMP-9, enzymes capable of degrading all macromolecular constituents of the extracellular matrix,79 has been shown to occur in plaque macrophages through a PGE2-cAMP dependent pathway.80 Increased expression of enzymatically active MMP-2 and MMP-9 has been reported in vulnerable regions of unstable carotid plaques81 in association with macrophages.82 Thus, localized increase of PGE2-dependent MMPs has the potential to cause acute plaque disruption in both the coronary and cerebral circulations.

The pathophysiologic role of functionally coupled COX-2/PGE synthase (PGES) has been recently supported by the demonstration that type 1 microsomal PGES (mPGES-1) expression is markedly induced by proinflammatory stimuli in vascular cells and is downregulated by dexamethasone,83 with concordant changes in COX-2 expression and delayed PGE2 generation.84,85 Thus, overexpression of functionally coupled COX-2/mPGES-1 in macrophages (Figure 2d) may dictate a predominant pathway of arachidonate metabolism, leading to increased biosynthesis of PGE2 and PGE2-dependent MMPs in the setting of human atherogenesis.

During the past 2 years, the concept of functional coupling among the PL-COX-PGH isomerase enzymes has gained experimental support.86 This model implies that initial formation of PGE2 involves preferential coupling between constitutively expressed cytosolic (c) PLA2, COX-1, and PGES. Under conditions favoring the induction of COX-2 and mPGES, formation of PGE2 involves coupling between cPLA2 and the latter enzymes. When exposure to receptor ligands is enduring and intense, the inducible, secreted (s) PLA2 isozyme begins to participate, creating an amplification loop to align arachidonic acid availability with the sustained capacity for prostanooid biosynthesis by inducible COX-2 and PGES. Furthermore, sPLA2 is responsible not only for delayed PGE2 production but also for direct COX-2 gene induction.87 The specific transmembrane signaling pathway(s) by which persistent stimuli (ie, ox-LDL, hyperglycemia, etc) may influence COX-2 expression in human plaque macrophages and smooth muscle cells are not yet completely elucidated. The recent demonstration88,89 that RAGE (receptor for advanced glycation end products [AGEs]) may upregulate COX-2 expression in plaque macrophages is interesting in this context. Thus, upregulation of RAGE is involved in sustaining MMP production by macrophages in atherosclerotic plaques of diabetic patients, most likely through enhanced signaling via PGE2.

In addition to COX-2, other metabolic pathways that use arachidonic acid as a substrate exist in human plaque macrophages and smooth muscle cells. In particular, the fatty acid–CoA ligase (FACL) 4 converts fatty acids to fatty acyl-CoA esters, and competes with COX-2 for the same substrate. In a recent study,89 we examined the expression level and localization of FACL4 in human carotid plaques and compared it with COX-2. We found that expression of FACL4 is significantly reduced in unstable plaques compared with stable plaques, suggesting that FACL4 could be a protective gene against the progression of atherosclerotic plaques toward instability.

Moreover, it should be noted that COX-2 is but an intermediate enzyme in the oxygenation of arachidonic acid, and that its product, PGH2, is further metabolized by other isomerases to various prostanooids (Figure 1). Thus, the relative abundance of a specific prostanooid is the result of the expression and activity of its specific synthase, and the coordinated induction of mPGES-1 and COX-2 in macrophages may lead in turn to a shift in arachidonic acid metabolism from the production of other prostanooids to the preferential synthesis of PGE2.91 We have recently suggested...
that the overexpression of COX-2 and mPGES-1 in the face of low levels of lypocalin-type PGD synthase (L-PGDS) may dictate a preferential pathway of arachidionate metabolism leading to increased biosynthesis of PGE\textsubscript{2}-dependent MMP-9 in human carotid plaques.\textsuperscript{92} By contrast, COX-2 overexpression in the presence of high levels of L-PGDS is associated with a stable plaque phenotype,\textsuperscript{92} possibly through generation of PGD\textsubscript{2} and 15d-PGJ\textsubscript{2}, compounds with anti-inflammatory properties. 15d-PGJ\textsubscript{2} is detectable as a minor product of COX-2 activity in human urine.\textsuperscript{93} Because proinflammatory stimuli may have different and opposing effects on PGDS and PGES gene expression,\textsuperscript{91} identification of the precise mechanisms of PGDS and PGES regulation may be critical to developing novel preventive strategies against atherosclerosis.

An additional layer of complexity is represented by the fact that there may be \( \geq 1 \) G protein–coupled receptor that transduces the effects of the same prostanoid (Figure 1).\textsuperscript{94} Moreover, activation of the same receptor (eg, EP4) may lead to an anti-inflammatory response during the early phase of atherosclerosis,\textsuperscript{95} while contributing to the progression of atherosclerotic plaque toward MMP-dependent instability in later stages of the disease.\textsuperscript{96}

Finally, we should consider the possibility that COX-2 gene variants in inflammatory cells could alter enzyme expression levels or activity, thereby influencing prostanooid biosynthesis. A single nucleotide polymorphism (\(-765G>C\)) in the COX-2 promoter has been reported to be associated with significantly lower promoter activity as compared with the \(-765G\) allele.\textsuperscript{97,98} Thus, the evidence that MMP production in carotid plaques depends, at least in part, on the induction of COX-2\textsuperscript{27} would predict that the \(-765C\) variant of the COX-2 gene may represent a protective genotype against atherothrombosis, possibly through plaque stabilization consequent to reduced PGE\textsubscript{2} production. To test this hypothesis, we have recently performed a case-control study in 1441 high-risk patients, 864 with and 577 without previous myocardial infarction or noncardioembolic ischemic stroke.\textsuperscript{99} The prevalence of \(-765G>C\) was significantly lower (\( P<0.0001\)) among cases than among controls. Expression of COX-2 and PGE\textsubscript{2}-dependent MMPs was significantly lower in carotid plaques from patients carrying the \(-765C\) allele, whereas the \(-765G>C\) polymorphism did not affect PGI\textsubscript{2} biosynthesis and endothelium-dependent vasodilation in vivo. Among subjects carrying the \(-765GC\) and \(-765CC\) genotypes, the odds ratio for having a myocardial infarction or stroke was significantly reduced compared with that for patients carrying the \(-765GG\) genotype.\textsuperscript{99}

### Pharmacological Modulation of COX-2

**Aspirin, Nonsteroidal Anti-inflammatory Drugs, and Coxibs**

When used at low doses (ie, 75 to 100 mg) administered once daily, aspirin is a relatively selective inhibitor of platelet COX-1, by virtue of its COX-isomerase selectivity and long dosing interval vis-à-vis its short half-life.\textsuperscript{1,48} Permanent inactivation of platelet COX-1 by aspirin is associated with reduced risk of myocardial infarction, ischemic stroke, and vascular death in randomized trials involving high-risk patients.\textsuperscript{1,48} However, in trials involving low-risk subjects, the only detectable effect of long-term aspirin administration was a reduced risk of nonfatal myocardial infarction.\textsuperscript{1,48}

Aspirin-insensitive TXA\textsubscript{2} biosynthesis has been described in patients with unstable angina,\textsuperscript{100-102} as well as in patients with poststroke dementia.\textsuperscript{103} Both COX-2 expression in inflammatory cells endowed with TX-synthase, and in newly formed platelets\textsuperscript{51} could account for TXA\textsubscript{2} biosynthesis in these settings. The clinical relevance of aspirin-resistant TXA\textsubscript{2} biosynthesis has been explored by Eikelboom et al,\textsuperscript{104} who performed a nested case-control study of baseline urinary TXA\textsubscript{2} metabolite excretion in relation to the occurrence of major vascular events in aspirin-treated high-risk patients enrolled in the HOPE trial. After adjustment for baseline differences, the odds for the composite outcome of myocardial infarction, stroke, or cardiovascular death in randomized trials involving high-risk patients enrolled in the HOPE trial. After adjustment for baseline differences, the odds for the composite outcome of myocardial infarction, stroke, or cardiovascular death in these studies was significantly lower (RR of 1.10 (95% CI, 1.02 to 1.19) for the association between NSAID use and myocardial infarction (García Rodríguez, personal communication, 2003). However, individual pharmacokinetic and/or pharmacodynamic features of some NSAIDs (eg, naproxen) have been associated with observational evidence of a cardioprotective effect, the size of which is substantially uncertain (an overview of 8 studies of naproxen use and myocardial infarction suggests a RR of 0.88 with 95% CI of 0.81 to 0.96; García Rodríguez, personal communication, 2003). Initiation of NSAID therapy may double the risk of developing heart failure in susceptible individuals, eg, those with hypertension or diabetes.\textsuperscript{105}

Highly selective inhibition of COX-2 in arthritic patients at relatively low cardiovascular risk (ie, \(<1\% per year\)) was not associated with a different rate of major vascular events compared with placebo or nonselective inhibition of COX-1 and COX-2 with nonnaproxen NSAIDs.\textsuperscript{107} However, in the randomized comparisons of rofecoxib versus naproxen (largely in patients with rheumatoid arthritis), a significantly different rate of vascular events (and, in particular, nonfatal myocardial infarction) was apparent between the two.\textsuperscript{107} Both the results of the VIGOR study and the metaanalysis of phase II through IV rofecoxib trials are compatible with the observed difference in vascular events, reflecting some cardio-protective effect of naproxen (possibly optimized by higher compliance with the bid regimen in the context of a randomized trial than in a real-life setting, as reflected by observational studies) plus the play of chance.\textsuperscript{107} However, a cardiovascular hazard resulting from COX-2 inhibition in the face of an independent predisposition to arterial thrombosis cannot be excluded. Potential variables contributing to different COX-2–dependent effects would include the daily dose of the inhibitor determining the extent of COX-2 inhibition, the half-life and dosing interval of the inhibitor determining the duration of COX-2 inhibition, and the patient’s substrate,
inasmuch as the importance of COX-2-dependent PGI₂ biosynthesis is likely to vary in different clinical settings.

No sizable study has compared a highly selective COX-2 inhibitor to a nonselective NSAID in aspirin-treated high-risk patients. It is likely that the hemodynamic consequences of vascular COX-2 inhibition by traditional NSAIDs or coxibs will be comparable in this setting, and largely determined by the extent and duration of COX-2 inhibition in the vasculature. The ongoing TARGET study has recruited ~4500 aspirin-treated (thus, presumably at high cardiovascular risk) arthritic patients who were randomized to receive 1-year treatment with lumiracoxib, ibuprofen, or naproxen. A meta-analysis of all the coxib trials, involving 5 different drugs and ~100 000 patient-years of exposure, is likely to provide some reliable answers to the various questions raised by the limited information available on each individual coxib, and may suggest working hypotheses for further investigation.

It is important to emphasize that none of the existing coxib trials has addressed those clinical settings in which COX-2 inhibition in high-risk aspirin-treated patients might actually be beneficial because of mechanistic considerations developed in the present review. Intervention with appropriate prostanoid receptor (eg, TP) antagonists might provide a more specific pharmacological approach to test this hypothesis. The main determinants and sources of variability in the cardiovascular read-outs of COX-2 inhibition are outlined in Figure 3.

**Figure 3.** Variables that may influence the cardiovascular read-out of COX-2 inhibition in an individual patient. Pharmacokinetic features, such as half-life of the drug, and pharmacodynamic features, such as its selectivity for the COX-2 isozyme, are intrinsic to the COX inhibitor. Moreover, intrinsic features of the patient will influence the interaction of COX-2 inhibition with preexisting risk factors for drug-dependent adverse effects (eg, heart failure) or COX-2-dependent pathophysiological mechanisms (eg, aspirin-insensitive TXA₂ biosynthesis) that may lead to a beneficial effect. Significant interindividual variability arises from several sources, including genetic variants of drug metabolizing enzymes; COX-2 gene variants and variable cellular pattern of COX-2 expression; variable pattern of expression of enzymes that are upstream and downstream of COX-2, as discussed in the text.

**Pharmacokinetics**

- Dose of the COX-2 inhibitor
- Plasma concentration

**Pharmacodynamics**

- COX-2 inhibition
- Clinical Read-Out

**Patient's substrate**

- Inter-individual variability

It is well known that angiotensin (Ang) II promotes several critical processes in atherogenesis. In particular, Ang II may induce the expression of COX-2 in vascular cells and influence the extracellular matrix turnover by regulating the activity of PGE₂-dependent MMPs. Notably, these effects are mediated by Ang II type 1 (AT₁) receptors, as reflected by in vitro studies using selective AT₁ receptor antagonists. Thus, blockade of the AT₁ receptor could contribute to plaque stabilization by inhibiting COX-2/mPGES-1 expression and the cascade of downstream events outlined above.

We have recently observed downregulation of COX-2/mPGES-1 expression in symptomatic carotid lesions after irbesartan (a selective AT₁ receptor antagonist) therapy, and provided evidence that this effect is associated with a stable plaque phenotype, by reducing inflammatory infiltrate and ox-LDL concentration, increasing interstitial collagen content, and suppressing MMP generation (data not shown).

Our results are consistent with recent studies demonstrating the ability of AT₁ antagonists in reducing the development of early atherosclerosis in monkeys with diet-induced hypercholesterolemia, as well as the inflammatory status in patients with premature atherosclerosis.

**Conclusions**

Experimental and clinical tools developed during the past 10 years have allowed us and other investigators to characterize variable patterns of COX-2 expression in the major cellular players of atherothrombosis and to hypothesize a role for...
COX-2–derived prostanooids in vascular disease progression and its thrombotic complications. The results of morphological, pharmacological, and genetic studies of the human carotid plaque model reviewed in this article are consistent with the hypothesis that downregulation of COX-2 expression in inflammatory cells may protect against atherothrombosis in high-risk aspirin-treated patients. However, the multifaceted aspects of prostanooid biology as well as the critical role played by COX-2–derived PG12 in maintaining systemic hemodynamics in the setting of inadequate circulatory volume should be considered when evaluating the potential benefits and risks of COX-2 inhibition. Intervention with selective prostanooid receptor antagonists might provide additional mechanistic insight. Moreover, the complexity of potential regulatory sites upstream and downstream of COX-2 expression should be emphasized when interpreting the results of human studies. Thus, the functional and clinical read-outs of COX-2 expression and inhibition may be importantly modulated by the variable expression of upstream enzymes utilizing arachidonic acid as a substrate, downstream PGH-isomerases that may preferentially couple to COX-isozymes in different cell types, as well as the diversity of pathophysiological settings with variable COX-2 dependence of platelet activation and vascular reactivity (Figure 3).

An integrated approach based on genetic, biochemical, and pharmacological profiling will provide further mechanistic insight into the role of the COX-2 pathway in atherothrombosis, characterize the determinants of the cardiovascular response(s) to COX-2 inhibitors, and identify novel targets for pharmacological intervention upstream or downstream of COX-2 expression.

Acknowledgments

The expert editorial assistance of Daniela Basilico is gratefully acknowledged. The studies reviewed in this paper were supported by grants from the Italian Ministry of Research and Education to the Center of Excellence on Aging of the University of Chieti and by a FIRB Project.

References


51. Caughey GE, Cleland LG, Pengils PS, Gamble JR, James MJ. Roles of cyclooxygenase (COX)-1 and COX-2 in prostanoic production by


Cyclooxygenase-2 Expression and Inhibition in Atherothrombosis
Francesco Cipollone, Bianca Rocca and Carlo Patrono

Arterioscler Thromb Vase Biol. 2004;24:246-255; originally published online October 30, 2003; doi: 10.1161/01.ATV.0000104005.92603.f2

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/24/2/246

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/