Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

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Arterioscler Thromb Vasc 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

Print ISSN: 1079-5642. Online ISSN: 1524-4636

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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.[1] 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.[2] These include D, E, F, and I prostaglandins (PGs) and thromboxane (TX) A_2, collectively termed prostanoids (Figure 1). Prostanoid biosynthesis involves 3 sequential enzyme-catalyzed 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, PGH_2; and (3) enzymatic rearrangement of PGH_2 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[3] (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.[4]

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.

Received July 18, 2003; revision accepted October 16, 2003.

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Arterioscler Thromb Vasc Biol. **is available at http://www.atvbaha.org** **DOI: 10.1161/01.ATV.0000104005.92603.f2**

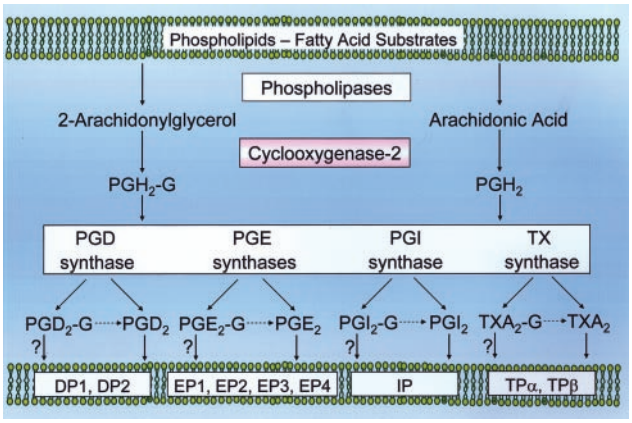

Figure 1. Production and actions of prostanoids originating via the COX-2 pathway. Arachidonic acid, a 20-carbon polyunsaturated fatty acid, is released from the sn2 position in membrane phospholipids by PLA_2 ($cPLA_2$ and $sPLA_2$), which is activated by diverse stimuli. Arachidonic acid is converted by cPGH synthases, which have both COX and hydroperoxidase activity, to the unstable intermediate PGH_2. PGH synthases are colloquially termed COXs and exist in 2 isoforms, COX-1 and COX-2. PGH_2 is converted by tissue-specific isomerases to multiple prostanoids. The conversion of PGH_2 to PGE_2 is catalyzed by 2isoforms of the enzyme PGES (and 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; DPs, PGD_2 receptors; EPs, PGE_2 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 phospolipases in a stimulus-induced manner. COX-2 can catalyze their oxygenation to provide PGH_2 glycerol ester (PGH_2-G) and PGH_2-ethanolamide (data not shown). mPGES catalyzes the isomerization of PGH_2-G to PGE_2 glycerol ester (PGE_2-G) at rates approaching those observed with PGH_2. Metabolism of 2-AG into TXA_2, PGD_2, and PGI_2 glycerol esters has also been described.[3]

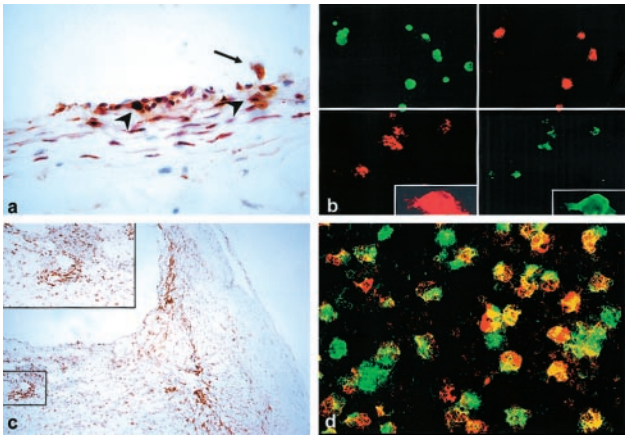

Figure 2. COX-2 expression in the different cellular players of atherothrombosis. a, COX-2 expression in monocytes during homing to the sub-endothelium. Note the positivity for COX-2 not only in monocytes/macrophages migrated into the vessel intima (arrowheads) but also in monocytes still adherent to 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 (5×) showing staining for COX-2 in the shoulder of a vulnerable plaque. At higher magnification (63×) (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.

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.[5,6] Despite the widely recognized role of COX-2 in human inflammatory disorders,[7] 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[8] observed in initial atherosclerotic lesions,[9] allowing subsequent migration of bloodborne cells into the arterial intima. COX-2 has been detected in the fatty streaks of both humans and mice.[10,11] Monocyte adhesion to activated endothelial cells in the presence of oxidized-LDL (ox-LDL) and interleukin (IL)-1 is enhanced by COX-2.[12] Adhesion of human monocytes to endothelial P-selectin, via the P-selectin glycoprotein ligand-1, rapidly induces COX-2 mRNA in monocytes[13,14] (Figure 2a). ox-LDL and proatherogenic ILs involved in early phases, such as IL-1α/β, tumor necrosis factor-α (TNF-α) or CD40 ligand,[15,16] alone or in combination, potently induce COX-2 and PGE_2 synthesis in human monocytes or monocytic cell lines,[13,17–25] by stabilizing COX-2 mRNA or enhancing transcription through nuclear factor (NF)-κB or peroxisome proliferator-activated receptor-γ (PPAR-γ), as for ox-LDL.[17] On the other hand, IL-4, IL-6, or IL-1 receptor antagonists, considered antiatherogenic ILs,[15] downregulate monocytic COX-2.[26,27] TNF-α has been reported to have no effect[24] or to inhibit monocytic COX-2, depending on the length of exposure,[23] indicating that TNF-α may play dual roles in inflammation[28] 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)[29] and by the indomethacin-induced reduction of LDL-dependent chemotaxis.[30] The kinetics of these phenomena strongly suggests COX-2 involvement; nevertheless, experiments using selective inhibitors are lacking.

A proatherogenic role for monocytic 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 hemopoietic cells.[11] 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.[31,32] Analyses of aortic lesions were performed at different time points in the 3 studies: 8 weeks in the study of Burleigh et al,[11] and 16 and 26 weeks in the study of Olesen et al[32] and Pratico et al,[31]

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 TXA_2 production by COX-2. We have recently detected enhanced biosynthesis of the potent vasoconstrictor LTC_4 during the acute phase of unstable angina, and the ability of glucocorticoids to down-regulate this phenomenon.[34] In addition, Mehrabian et al[35] 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 TXA_2 and PGE_2 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, TXA_2 seems prevalent from COX-2 activity of PMNs, whereas PGE_2 is predominant from monocytic COX-2. TXA_2 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-$PGF_2\alpha$[47] 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 PGH_2-isomerase. TXA_2 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 prostanoid 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 megakaryocytes[51] (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. PGE_2 represents the main product of platelet COX-2 activity, although under high platelet turnover, a detectable amount of TXA_2 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 PGE_2 by altering their deformability and volume,[55,56] can release AA through a specific PLA_2,[56] and can further metabolize it via the COX pathway, primarily to PGE_2 (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 prostanoids 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.[13,14,58,59] Interestingly, circulating leukocyte-platelet aggregates have been observed in unstable angina patients.[60] Under these circumstances, COX-2 upregulation might provide PGH_2 to platelet TX-synthase,[61] thus facilitating aspirin-insensitive TXA_2 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 PGI_2 biosynthesis after coxib administration to healthy subjects.[65] PGI_2 is considered antiatherogenic, causing vasodilation and platelet inhibition. COX-2–dependent PGI_2 production by endothelial cells has been reported to be modulated in vitro by laminar shear stress,[66] thrombin,[67] microparticles shedded 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 pro-inflammatory COX-2 in leukocytes and macrophages.

Interestingly, PGI_2 synthesis from human aorta samples decreases as a function of progressing atherosclerotic lesions, whereas PGE_2 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 PGI_2 metabolite excretion was not associated with enhanced platelet activation, as reflected by TXA_2 metabolite excretion, in atherosclerotic patients treated with COX-2 inhibitors,[73] 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 PGI_2 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 macrophages[77,78] (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[11,77] (Figure 2c), thus suggesting that macrophage COX-2 may be downregulated in mature foam cells. Whereas endothelial cells predominantly release PGI_2, macrophages synthesize an array of prostanoids, including PGE_2, 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 PGE_2-cAMP dependent pathway.[80]

Increased expression of enzymatically active MMP-2 and MMP-9 has been reported in vulnerable regions of unstable carotid plaques[81] in association with macrophages.[82] Thus, localized increase of PGE_2-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 PGE_2 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 PGE_2 and PGE_2-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 inaugural formation of PGE_2 involves preferential coupling between constitutively expressed cytosolic (c) PLA_2, COX-1, and cPGES. Under conditions favoring the induction of COX-2 and mPGES, formation of PGE_2 involves coupling between $cPLA_2$ and the latter enzymes. When exposure to receptor ligands is enduring and intense, the inducible, secreted (s) PLA_2 isozyme begins to participate, creating an amplification loop to align arachidonic acid availability with the sustained capacity for prostanoid biosynthesis by inducible COX-2 and PGES. Furthermore, $sPLA_2$ is responsible not only for delayed PGE_2 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 demonstration[88,89] 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 PGE_2.

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,[90] 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, PGH_2, is further metabolized by other isomerases to various prostanoids (Figure 1). Thus, the relative abundance of a specific prostanoid 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 prostanoids to the preferential synthesis of PGE_2.[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 arachidonate metabolism leading to increased biosynthesis of PGE_2-dependent MMP-9 in human carotid plaques.[92] By contrast, COX-2 overexpression in the presence of high levels of L-PGDS is associated with a stable plaque phenotype,[92] possibly through generation of PGD_2 and 15d-PGJ_2, compounds with anti-inflammatory properties. 15d-PGJ_2 is detectable as a minor product of COX-2 activity in human urine.[93] Because proinflammatory stimuli may have different and opposing effects on PGDS and PGES gene expression,[91] identification of the precise mechanisms of PGDS and PGES regulation may be critical to developing novel preventive strategies against atherothrombosis.

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

Finally, we should consider the possibility that COX-2 gene variants in inflammatory cells could alter enzyme expression levels or activity, thereby influencing prostanoid 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.[97,98] Thus, the evidence that MMP production in carotid plaques depends, at least in part, on the induction of COX-2[77] 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_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.[99] The prevalence of -765G>C was significantly lower ($P<0.0001$) among cases than among controls. Expression of COX-2 and PGE_2-dependent MMPs was significantly lower in carotid plaques from patients carrying the -765C allele, whereas the -765G>C polymorphism did not affect PGI_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.[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-isoform selectivity and long dosing interval vis-à-vis its short half-life.[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.[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.[1,48]

Aspirin-insensitive TXA_2 biosynthesis has been described in patients with unstable angina,[100–102] as well as in patients with poststroke dementia.[103] Both COX-2 expression in inflammatory cells endowed with TX-synthase, and in newly formed platelets[51] could account for TXA_2 biosynthesis in these settings. The clinical relevance of aspirin-resistant TXA_2 biosynthesis has been explored by Eikelboom et al,[104] who performed a nested case-control study of baseline urinary TXA_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 increased with each increasing quartile of 11-dehydro-TXB_2 excretion, with patients in the upper quartile having a 1.8-times higher risk than those in the lower quartile.[104]

Nonselective reversible inhibition of COX-1 and COX-2 by traditional nonsteroidal anti-inflammatory drugs (NSAIDs) is not associated with clear evidence of a protective effect against myocardial infarction[105] or stroke.[106] In fact, a recent overview of 8 published observational studies reported an odds ratio 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.[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.[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.[107] Both the results of the VIGOR study and the metanalysis of phase II through IV rofecoxib trials are compatible with the observed difference in vascular events, reflecting some cardioprotective 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.[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,

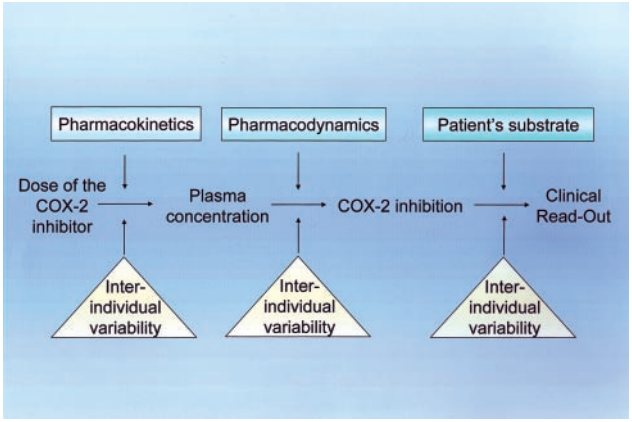

Figure 3. Variables that may influence the cardiovascular read-out of COX-2 inhibition in an individual patient. Pharmacokinetic features, such as half-life of the drug, and pharmacodynamic features, such as its selectivity for the COX-2 isoform, are intrinsic to the COX inhibitor. Moreover, intrinsic 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 pathophysiologic mechanisms (eg, aspirin-insensitive TXA_2 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.

inasmuch as the importance of COX-2-dependent PGI_2 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.

Statins

After the characterization of functionally coupled COX-2/mPGES-1 as an important determinant of atherosclerotic plaque instability,[77] we have provided evidence for the critical involvement of these enzymes in the process of carotid plaque stabilization induced by statin therapy.[108] In particular, concordantly higher expression of COX-2, mPGES-1, MMP-2, and MMP-9 was found in plaques obtained from the "culprit" carotid lesions of symptomatic patients randomized to American Heart Association step 1 diet alone compared with specimens obtained from patients randomized to simvastatin. In this study, macrophages were significantly more abundant in plaques obtained from patients randomized to diet alone, always outnumbered lymphocytes and represented the major source of COX-2/mPGES-1, MMP-2, and MMP-9.[108]

The results observed with simvastatin are consistent with recent in vitro evidence[109] demonstrating that atorvastatin may reduce inflammation by decreasing COX-2 expression in smooth muscle cells. However, mevastatin and lovastatin have been reported to upregulate COX-2 expression in the same cells,[110] thus suggesting that different statins may variably affect the complex signal transduction pathways of COX-2 expression in smooth muscle cells.

The hypothesis that COX-2/mPGES-1 downregulation by statin is largely dependent on the reduction in plaque cholesterol is supported by in vitro experiments with mevalonate[108] and by the observation that lower COX-2/mPGES-1 expression was associated with comparable reduction in plaque oxLDL content. However, further studies directly comparing statins with other lipid-lowering strategies are necessary validate this hypothesis.

Angiotensin II Receptor Blockers

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[111,112] in vascular cells and influence the extracellular matrix turnover by regulating the activity of PGE_2-dependent MMPs.[113] Notably, these effects appear mediated by Ang II type 1 (AT_1) receptors, as reflected by in vitro studies using selective AT_1 receptor antagonists.[111] Thus, blockade of the AT_1 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 AT_1 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 AT_1 antagonists in reducing the development of early atherosclerosis in monkeys with diet-induced hypercholesterolemia,[114] as well as the inflammatory status in patients with premature atherosclerosis.[115]

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 prostanoids 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 prostanoid biology as well as the critical role played by COX-2–derived PGI_2 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 prostanoid 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 pathophysiologic 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 “G. d’Annunzio” and to a FIRB Project.

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