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EDITORIAL COMMENT

Cyclooxygenase-2 Inhibitors and Atherosclerosis*

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The report by Rott et al. (1) in this issue of the *Journal* adds to the concerns surrounding the cardiovascular effects of cyclooxygenase (COX)-2 inhibitors. However, their finding of an increased burden of atherosclerosis in the apolipoprotein E (apoE) knockout mouse treated with a selective COX-2 inhibitor must be seen in the context of other data suggesting that COX-2 contributes to lesion formation or has no effect whatsoever.

Prostaglandin (PG) generation and activity. Prostaglandins are generated from arachidonic acid by the enzyme COX, which has two isoforms: COX-1 and COX-2. The former is ubiquitously expressed and poorly inducible, whereas the latter is largely absent from normal cells but is induced by cytokines (2), growth factors (3), oxidized lipids (4), and free radicals (5), factors known to play a role in

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atherosclerosis. That said, there is evidence that COX-1 is inducible (6) and that COX-2 is expressed in normal subjects. For example, COX-2 inhibitors reduce PGI_2 formation in normal volunteers by up to 80% (7,8). Cyclooxygenases convert arachidonic acid to PG endoperoxides, which in turn are converted by a series of isomerases to the corresponding PG. Cells tend to express a predominant isomerase (prostacyclin synthase, thromboxane synthase, PGE synthase, PGD synthase) closely coupled with COX, which largely determines which product is generated. However, COXs can couple to different isomerases in the same cell (9); cells can express more than one isomerase (10); and there is evidence that endoperoxides generated in one cell type may be metabolized by isomerases of adjacent cells, so-called "transcellular metabolism" (11).

Prostaglandins mediate their effects through transmembrane G-protein–coupled receptors, several of which exist for each PG (12). For example, there are at least four different PGE-type receptors and two thromboxane A_2 (TXA_2) receptors (TP), the latter being alternatively spliced variants derived from a single gene (13). As these are cell-surface receptors, PGs presumably act in a paracrine or autocrine fashion. They also activate peroxisome proliferator-activated receptors (PPARs), nuclear membrane proteins that dimerize with other cell proteins to form transcription factors (14). In this way, PGs may act as intracellular signaling molecules and regulate gene expression (15). There are at least four types of PPARs (alpha, delta, gamma-1, and gamma-2) that exhibit differential sensitivity to PGs (14). In short, in diseased tissue composed of a variety of cells, a bewildering number of products with diverse functions are generated. Consequently, the effects of a COX inhibitor, acting high in this pathway, are likely to be complex.

PGs and atherosclerosis. Cyclooxygenase expression (16,17) and PG generation (17–19) are induced in patients with atherosclerosis. Cyclooxygenase-2 is expressed in the monocytes/macrophages and proliferating vascular smooth muscle cells that typify atherosclerotic lesions, in addition to endothelium (16,17). Patients with extensive disease have enhanced formation of TXA_2, a potent platelet activator and vasoconstrictor, largely derived from COX-1 in platelets (18). Formation of prostacyclin (PGI_2), a potent platelet inhibitor and vasodilator, is also increased largely through COX-2 (17,19). Thus, in atherosclerosis, COX-2 inhibition preferentially suppresses PGI_2 generation and spares TXA_2, as it also does in normal individuals (7,17). Theoretically, PGI_2 may limit the extent of platelet adhesion and activation at sites of vascular disease. For example, local delivery of prostacyclin synthase using an adenoviral vector reduces the platelet deposition seen following vascular injury (20). In addition, disruption of the PGI_2 receptor (IP) enhances the response to carotid injury in the mouse. This is largely dependent on TXA_2, as coincident disruption of the TP receptor prevents the amplification of vascular injury (21). Given that PGI_2 regulates the response to TXA_2 and COX-2 inhibition selectivity suppresses PGI_2, it follows that COX-2 inhibitors may enhance platelet activity. This provides a plausible explanation for the increased risk of myocardial infarction reported with the COX-2 inhibitor called rofecoxib (22).

COX-2 inhibitors and atherosclerosis. There have been reports that disruption of the IP receptor aggravates atherosclerosis in low-density lipoprotein receptor (LDLR) knockout mice (23); furthermore, it has been suggested that deletion of the IP receptor results in increased sensitivity to thrombotic stimuli (24). However, as platelets contribute to the development and progression of atherosclerosis, it follows that PGI_2 formed locally may suppress lesion formation by limiting platelet deposition. Of course, COX-2 generates products other than PGI_2, so the response to COX-2 inhibitors may be quite different from those seen when PGI_2 is selectively inhibited. It should also be emphasized that targeted gene deletion of the IP receptor may greatly exceed the effect achieved by COX-2 inhibitors, where PGI_2 generation is reduced but not ablated.

There are data demonstrating that COX-2 expressed in monocytes/macrophages contributes to the development of atherosclerosis in murine models (25) and to the expression

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of proteins, such as metalloproteinases, which contribute to plaque instability (26). In that case, inhibition of COX-2 would be expected to limit the extent of atherosclerosis. Indeed, Pratico et al. (23) reported a 30% reduction in atherosclerosis by the COX-2 inhibitor nimesulide. Administration of the COX-2 inhibitor rofecoxib was also shown to reduce atherosclerosis in a mouse model deficient in the LDLR (25). In contrast, selective inhibition of COX-2 had no effect on the restenosis that occurs following balloon angioplasty of the rat carotid artery, whereas selective inhibition of COX-1 reduced lesion formation, possibly reflecting an antiplatelet effect (27). A role for COX-1–mediated PGs is further supported by studies showing that nonselective COX inhibitors, such as aspirin and indomethacin, retard the development of atherosclerosis in apoE$^{-/-}$ mice (28) to a greater extent than a selective COX-2 inhibitor (25).

COX-2 and angiogenesis. Several PGs modulate angiogenesis and endothelial cell apoptosis, both of which are implicated in atherosclerosis (29). For example, PGE_2 and PGI_2 induce endothelial cell growth and new blood vessel formation (30), whereas TXA_2 and PGJ_2 have the opposite effect (31). Both COX isoforms have been shown to play a role in angiogenesis in several model systems. Selective inhibition of COX-2 inhibits the growth of angiogenic endothelial cells in vitro (32) and angiogenesis in animal models of inflammation (33), whereas COX-1 activity in endothelial cells modulates the angiogenesis that occurs around transplanted tumors (34). In cardiovascular disease, angiogenesis could have both beneficial and deleterious effects. Angiogenesis may contribute to the revascularization of ischemic tissues. On the other hand, angiogenesis in an atherosclerotic lesion might result in plaque expansion and vulnerability.

COX-2 as an anti-inflammatory gene. It is surprising, therefore, that Rott et al. (1) found that inhibition of COX-2 aggravated atherosclerosis in the apoE knockout mouse. The study was more complex, as the authors set out to examine the effect of COX-2 inhibition on infectivity of cytomegalovirus and coincidentally showed increased disease burden in animals treated with the COX-2 inhibitor, including those not infected with the virus. Although it would be reasonable to conclude that this reflected selective suppression of PGI_2 and an unopposed effect of TXA_2, the authors suggest as an alternative hypothesis the suppression of anti-inflammatory PGs, such as PGJ_2, and its metabolite 15-deoxy-delta^{12,14}-PGJ_2 (15d-PGJ_2) (35).

Although COX-2 is usually thought of as contributing to inflammation, some authors have suggested that COX-2 may also play an anti-inflammatory role. The hypothesis arises from observations with COX-2 inhibitors and in animals in which the COX-2 gene has been disrupted. Although inducing an anti-inflammatory effect acutely, inhibition of COX-2 or disruption of the COX-2 gene can aggravate inflammation chronically (36,37). For example, in a rat model of carrageenan-induced pleurisy, Gilroy et al. (37) found that COX-2 inhibitors, while suppressing inflammation initially, aggravated the inflammatory response at 48 h after the injection of the antigen. These findings have raised the possibility that COX-2 generates an anti-inflammatory product, possibly 15d-PGJ_2.

15d-PGJ_2 is one of a series of products derived nonenzymatically from PGD_2. Unlike other PGs, which are pro-inflammatory, 15d-PGJ_2 displays remarkable anti-inflammatory effects (35). Several mechanisms have been suggested, including induction of apoptosis in monocytes (38), activation of PPAR-gamma, and modification of p50, a subunit of the pro-inflammatory transcription factor, nuclear factor kappa-B (39,40). Indeed, 15d-PGJ_2 has been found by immunoblot analysis in atherosclerotic plaque (41). Given the structural similarities between different PGs, immunologic identification is fraught with problems, and more specific assays will be needed to demonstrate the formation of PGJ_2 and its metabolites. Highly specific assays based on mass spectrometric identification of the metabolites have shown that human cells are capable of generating PGJ_2 and its metabolites in an enzymatic fashion, which is hardly surprising since these products derive from PGD_2 (42). What is unclear, as yet, is whether sufficient amounts of the cyclopentenone PGs are generated and remain in an active conformation to have any biologic effect. Given their propensity to complex with peptides (e.g., glutathione) and proteins, this is still in doubt.

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