Unresolved Issues in the Role of Cyclooxygenase-2 in Normal Physiologic Processes and Disease

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Originally suggested to function mainly in inflammatory situations, recent data have implied important roles for the cyclooxygenase-2 isoenzyme in reproductive biologic processes, renal and neurologic function, and the antithrombotic activities of endothelial cells. As cyclooxygenase-2–specific inhibitors have recently become available as analgesic and anti-inflammatory drugs, a comprehensive view of this rapidly evolving field is necessary to anticipate both the potential therapeutic benefits and toxic effects associated with these agents.

Since the synthesis of aspirin 100 years ago, nonsteroidal anti-inflammatory drugs (NSAIDs) have become mainstays in the medical management of pain and inflammation.1 The common but perhaps not the only mechanism underlying NSAID activity, inhibition of the cyclooxygenase (COX) enzyme that catalyzes the initial step of arachidonic acid metabolism, became clear in the early 1970s,2 and served to encourage the development of an NSAID class that now includes over 30 compounds. Despite the capacity of these agents to suppress pain, inflammation, and fever,3 and the expansion of the use of these agents into one of the most widely used classes of drugs in the world, mechanism-based toxicity related to the suppression of the production of specific arachidonic acid metabolites in individual tissues and organs4 has limited unmitigated acceptance of these compounds. Moreover, this mechanism-based toxicity has stimulated research into the possibility that less toxic analgesic and anti-inflammatory agents could be developed.

Within the past 10 years, COX activity was found to be associated with 2 distinct isozymes, COX-1 and COX-2.1,5-7 Initial evidence suggested that COX-1 was expressed constitutively within many tissues and was thought to be responsible for homeostatic production of arachidonic metabolites. In contrast, COX-2 was thought not to be expressed normally, but to be rapidly induced in response to inflammatory stimuli and to be responsible for the large amounts of prostaglandin E2 (PGE2) and other arachidonic acid metabolites produced at inflammatory sites. This generated the hypothesis that the functions of the COX isoforms were mutually exclusive, with COX-1 involved in maintenance of the physiologic function of a variety of organs and COX-2 involved in pathophysiologic processes, including inflammation, pain, and fever. This hypothesis was so compelling that before it was rigorously tested, it served as the rationale to develop specific COX-2 inhibitors (agents that at therapeutic doses block the activity of COX-2 but not COX-1) with the expectation that these agents would have all of the anti-inflammatory and analgesic properties of standard NSAIDs, but lack the well-recognized toxic effects related to COX-1 inhibition.8,9 Clinical trials with 2 of these agents (Figure 1) have produced results consistent with this general paradigm and have led to the approval of these agents for the treatment for osteoarthritis (celecoxib, rofecoxib), rheumatoid arthritis (celecoxib), and acute pain (rofecoxib).10,11 However, newly emerging information has challenged some as-
pects of the original model, documenting much wider physiologic roles for both COX-1 and COX-2. This information has altered some aspects of the anticipated outcome of treatment with specific COX-2 inhibitors, but also expanded their potential therapeutic indications.

DISCOVERY OF COX-2

Although a multitude of NSAID actions have been proposed, the ability of NSAIDs to suppress inflammation and inflammatory pain results primarily from their inhibition of arachidonic acid metabolism and, specifically, PGE₂ production.¹⁴,¹⁵,¹⁶ Because arachidonic acid metabolites also maintain gastric mucosal integrity (PGE₂, prostacyclin) and platelet function (thromboxane A₂), as well as renal blood flow, especially in the face of volume contraction, inhibition of arachidonic acid metabolism also explains the mechanism-based toxicity of NSAIDs, including gastrointestinal ulceration and bleeding and diminished renal function.¹⁴,¹⁵-¹⁷

Also called prostaglandin H synthase, COX is the first enzyme in the prostanoïd biosynthetic pathway catalyzing the conversion of arachidonic acid to prostaglandin G₂ and then to prostaglandin H₂.¹⁵,¹⁶ Subsequent activity by a variety of other specific enzymes results in the characteristic array of arachidonic acid metabolites produced by individual cells and tissues. It is the regulated production of those specific metabolites that determines the unique and often opposing dominant effects of arachidonic acid metabolites at specific tissue sites. For example, platelet derived thromboxane favors platelet aggregation and thrombosis that is opposed by the vasodilatory effects of endothelial cell–derived prostacyclin.

In the late 1980s, it was shown that expression of COX activity could be markedly stimulated by interleukin-1 in fibroblasts and monocytes and inhibited by corticosteroids.²⁴-²⁶ This was important because prostaglandin production was previously thought to be determined only by the amount of arachidonic acid substrate present. Based on this work, the existence of 2 distinct forms of COX was proposed, one constitutive and one inducible.²⁵ Since that time, separate genes for the 2 isoenzymes have been cloned,²⁷-²⁹ and regulation and expression of the 2 proteins have been delineated, providing clues to their proposed distinct biological roles.²⁵-²⁷

The genes for the 2 COX isoforms are approximately 65% homologous in their coding regions and, as a result, the proteins are quite similar, with comparable enzymatic activities and substrate specificities. One potential difference in the enzymatic activities of the 2 isoforms is the source of the arachidonic acid substrate, with COX-2 using intracellular arachidonic acid and COX-1 using extracellular substrate.²⁵,³⁰-³¹ Soluble phospholipase A₂, produced by a variety of cells, seems to be important in providing extracellular arachidonic acid substrate for COX-1.²⁵,³⁰-³¹ As the amount of substrate might be an essential contributor of arachidonic acid metabolite production by COX-1, regulation of soluble phospholipase A₂ rather than COX-1 itself may provide the crucial influence of the metabolic activity of the COX-1 isoform.

One additional difference between COX-1 and COX-2 emerged from analysis of the 3-dimensional structures of the molecules.³²,³³-³⁵ A subtle difference in the structures of the hydrophobic channel leading to the active site of the COX-2 molecule has been identified, with a somewhat larger orifice and an additional pocket pointing away from the catalytic site. This has permitted the development of inhibitors that block the activity of COX-2, specifically at concentrations that have only minimal effects on COX-1.³⁶,³⁷ In general, COX-2 inhibitors differ from classic competitive inhibitors in that they require time to fit into the active site of the enzyme, after which their inhibitory effects may become persistent. Of note, the same compound may function as a competitive inhibitor of COX-1 at high concentrations and as a “timed inhibitor” of COX-2 at markedly lower concentrations owing to the unique configuration of the hydrophobic channel leading to the active site of the COX-2 isoform. Of importance, the differences between the mechanisms of inhibition of COX-1 and COX-2 can influence the estimation of the activity of a putative inhibitor when analyzed with only isolated enzymes or intact or broken cells.

THE HOMEOSTATIC VS PROINFLAMMATORY THEORY OF COX ACTIONS

Analysis of the expression of COX-1 using monoclonal antibodies and molecular probes has documented that this isoform is expressed constitutively in many cells and tissues.³⁶ Of importance, in certain tissues and cells such as the normal gastric mucosa and the platelet, COX-1 is the only isoform expressed. In the gastric antrum, local production of PGE₂ and endothelial cell–derived prostacyclin synthesized via the action of COX-1 promotes vasodilatation, thereby promoting the maintenance of mucosal integrity.²¹-²³ Similarly, in the kidney, COX-1 is important in producing vasodilatory prostaglandins that maintain renal blood flow and the glomerular filtration rate, especially during periods of systemic vasoconstriction.³⁷,³⁸ Finally, in platelets, the action of COX-1 is essential for the production of thromboxane A₂ that promotes platelet aggregation.³⁹ These findings stimulated the concept that the major, if not the only, function of COX-1 was to maintain homeostasis and promote specific physiologic activities.

In contrast to the constitutive expression of COX-1 and its putative role in homeostatic regulation of physiologic processes, the COX-2 enzyme was initially noted to be undetectable in most normal tissues and cells.⁵,⁷,³⁶ However, when a va-
variety of cells such as macrophages and endothelial cells were challenged with various inflammatory mediators, COX-2 expression was rapidly induced. Moreover, at sites of inflammation such as the rheumatoid synovium, COX-2 was dramatically up-regulated. Finally, in animal models of inflammation, COX-2 messenger RNA and protein, but not COX-1, were dramatically up-regulated at the inflammatory site by the evoking stimulus and just before the marked increase in local prostaglandin production and clinical manifestation of inflammation. This evidence suggested the hypothesis that COX-2 was an inducible enzyme that was markedly up-regulated at sites of inflammation and accounted for the increased production of arachidonic acid metabolites locally and the resultant vasodilation, edema, and pain.

This information provided the basis for the hypothesis that COX-1 was involved in cellular “housekeeping functions” necessary for normal physiologic activity, whereas COX-2 acted primarily at sites of inflammation to amplify pain and pro-inflammatory manifestations. The clinical corollary to this hypothesis was that highly specific inhibition of COX-2 would exert beneficial anti-inflammatory and analgesic effects without influencing the important physiologic functions of COX-1. Since all currently available NSAIDs inhibit both COX-1 and COX-2 to varying degrees by competing with arachidonate for binding to the active site of the enzyme, this line of reasoning suggested that the toxicity of these agents might be related to their capacity to inhibit COX-1, whereas their analgesic, anti-inflammatory and antipyretic effects might depend on their ability to inhibit COX-2. The potential of segregating the “good” from the “bad” actions of NSAIDs stimulated the search for agents that inhibited COX-2 specifically.

Evolving knowledge of the biologic function of COX-1 and COX-2 has suggested that the initial paradigm is an oversimplification. Although COX-2 is induced at sites of inflammation, a critical role for COX-2 in a number of other physiologic processes has emerged. Moreover, in certain circumstances COX-1 has been shown to be induced and to play a protective function, or to contribute to inflammatory responses. Thus, a more complex interplay of COX-1 and COX-2 in physiologic and pathophysiologic processes has emerged with certain unexpected outcomes resulting from targeted disruption or inhibition of specific COX isoforms. Moreover, a role for COX isoforms in unanticipated physiologic or pathophysiologic processes has emerged, suggesting unexpected therapeutic opportunities or consequences of specific COX-2 inhibition.

**EMERGING COMPLEXITY: DIVERSE PHYSIOLOGIC AND PATHOPHYSIOLOGIC ROLES FOR COX-1 AND COX-2**

Emerging information suggests that both COX-1 and COX-2 play broad and complex physiologic and pathophysiologic roles. Animal data, for example, demonstrate that COX-2 is expressed constitutively in the kidney and brain and can be induced by physiologic stimuli in the kidney, brain, ovary, uterus, cartilage, and bone. Conversely, COX-1 can be induced in response to injury, for example in the crypt cells of the small intestine after radiation injury, and play a role in regeneration. Cyclooxygenase-2 seems to play an important role in a number of essential physiologic functions such as ovulation and implantation, whereas COX-1 may play a critical role in inflammation, especially when it is induced by extracellular arachidonic acid or when it occurs in the skin. These findings have provided a more complex model of the interplay of COX-1 and COX-2 in both normal physiologic processes and in pathophysiologic conditions than the homeostasis vs inflammation paradigm of COX-1 and COX-2 action originally suggested.

**Renal Function**

Cyclooxygenase-1, expressed in the vasculature, glomeruli, and collecting ducts of the kidney, seems to produce vasodilator prostaglandins that maintain renal plasma flow and glomerular filtration rate especially during conditions of angiotensin-stimulated systemic vasoconstriction. The NSAIDs, known to have multiple clinical effects on kidney function are thought to block this COX-1 protective response and lead to renal ischemia and functional damage in some individuals.

Recent studies have suggested that COX-2 may also play a role in the development of the renal cortex and in maintaining kidney function. Mice that do not express COX-2 because of targeted gene disruption (COX-2–null mice) show severe disruption of kidney development. Cyclooxygenase-2 is expressed in the interstitial cells of the medulla of the rabbit kidney and in the macula densa and the thick ascending loop of Henle in the rat kidney. Moreover, recent work suggests that COX-2 is also expressed in the human kidney—not in the macula densa, but rather in the podocytes of the glomerulus and the endothelial cells of arteries and veins.

Chronic sodium deprivation or experimental hyperfiltration states increase COX-2 expression in the rat kidney, suggesting that the prostaglandin produced by COX-2 may function to increase sodium reabsorption in response to volume contraction or hyperfiltration that may occur with progressive renal failure. Renal COX-2 is also up-regulated in the rat by long-term administration of angiotensin-converting enzyme inhibitors or type 1 angiotensin 2 receptor antagonists, suggesting feedback inhibition of COX-2 expression by the renin-angiotensin system. Moreover, a specific COX-2 inhibitor blocked the increase in plasma and kidney renin levels induced by captopril and also in a model of renovascular hypertension. As shown in Figure 2, these results suggest that COX-2 plays a role in the regulation of renin production. Thus, in the rat, angiotensin 2 appears to down-regulate COX-2 expression, whereas COX-2 is involved in the increased production of renin in response to inhibition of angiotensin 2 production.

In normal humans, specific COX-2 inhibitors induce a tran-
sient sodium retention associated with a marked decrease in 6-keto prostaglandin F₁α, excretion, a measure of renal prostacyclin production, but no alteration in glomerular filtration rate. These results are consistent with the conclusion that a major fraction of renal prostacyclin production is dependent on COX-2 activity, presumably in the renal vasculature, and that this may contribute to renal sodium balance independent of an effect on renal hemodynamics. Of importance, glomerular filtration rate in normal subjects, even the normal elderly, does not seem to depend on renal COX-2 function. Whether this is also the case in individuals with intrinsic renal disease or those with hypertension or volume contraction remains to be determined.

Gastrointestinal Tract Integrity

Cyclooxygenase-1 is the only COX isofrom identified in the gastric mucosa of normal animals, including humans, and is intimately involved in protecting the stomach from erosions and ulceration. As a result of inhibiting COX-1, all currently available traditional NSAIDs impose a risk of gastric ulceration and the major complications of gastrointestinal bleeding, perforation, and obstruction. Gastrointestinal bleeding caused by NSAIDs seems to relate to 2 events: inhibition of platelet COX-1 activity that increases the tendency to bleed and inhibition of gastric COX-1 that increases the likelihood of ulceration. The net result is a relative risk for gastrointestinal bleeding of approximately 4 for currently available NSAIDs. Since COX-2 is not detectable in the normal gastric mucosa nor in the platelet inhibition of COX-2 would not be expected to impose a risk of gastric ulceration or bleeding. However, COX-2 is expressed during the acute stages of gastric erosion and ulceration in animal models and might play a role in facilitating ulcer healing. Therefore, COX-2-specific inhibitors may increase the risk of major gastrointestinal adverse effects not by increasing the likelihood of developing an ulcer or bleeding, but by decreasing ulcer healing induced by other stimuli such as Helicobacter pylori or concomitant aspirin administration. The potential clinical impact of this effect of specific COX-2 inhibition has not yet been reported in clinical trials and, therefore, the relative risk of gastrointestinal bleeding associated with these agents is not certain.

Cyclooxygenase-2 may also play an important physiologic role in other parts of the gastrointestinal tract. In response to invasion by pathogenic microorganisms, epithelial cells express COX-2, which leads to increased prostaglandin production. This seems to play a protective role in the stimulation of the chloride and fluid flux that flushes bacteria from the intestine. Thus, COX inhibitors block the rapid intestinal secretion of fluid that accompanies the Salmonella infection of rhesus monkeys. Moreover, antibodies to PGE₂ block the accelerated production of chloride from bacterially infected intestinal cells. Together, the data indicate that invasion by pathogenic microorganisms leads to the production of COX-2 by intestinal cells, which catalyzes the production of PGE₂, which governs the chloride and fluid secretion involved in expelling the intestinal pathogen. The potential protective role of COX-2 in the intestine is emphasized by the fact that COX-2 levels are increased in inflammatory diseases such as ulcerative colitis, whereas selective inhibition of COX-2 may exacerbate inflammation in animal models of colitis. The exact role of COX-2 in maintaining intestinal integrity in humans remains unresolved, but specific COX-2 inhibitors could limit intestinal healing or diminish resistance to invasive microorganisms.

Nerve and Brain Function

Prostaglandin production plays a central role in the fever response and is thought to play a role in certain specific manifestations of brain function. The mechanism underlying the fever response seems to involve the COX-2 enzyme. In rats, intraperitoneal injection of lipopolysaccharide causes a marked fever response that temporally parallels COX-2 induction in the endothelial cells of the brain vasculature. This is thought to be mediated by interleukin-1β and perhaps other cytokines produced in response to lipopolysaccharides that stimulate brain endothelium. The resulting prostaglandins then act on temperature-sensing neurons in the preoptic area to produce the fever response. Cyclooxygenase-2–specific inhibition effectively blocks the fever response. Moreover, pyrexia in response to lipopolysaccharide stimulation does not occur in mice rendered COX-2 deficient by targeted gene disruption. By contrast, COX-1–deficient mice have a normal fever response.

Cyclooxygenase-2 also seems to play an essential role in neural development and adaptation. While early-stage brain formation seems to be internally crafted by developmentally induced neural genes and proteins, the final stages of brain maturation are more environmentally impacted by neural responses and synaptic activity and coincide with the local expression of COX-2 activity. Cyclooxygenase-2 is expressed most notably during ontogeny in the cortex and hippocampus. Throughout adult life, COX-2 may remain an important modulator of specific neural responses. Seizures, for example, strongly induce COX-2 in the postsynaptic dendritic arborization of excitatory neurons in major processing centers of the
brain.\(^{31,52}\) Associations between COX-2 induction and neural degeneration following stresses such as glutamate stimulation,\(^{33}\) seizures, and spreading depressive waves\(^{34}\) suggest that the role of COX-2 and arachidonic acid metabolites produced may be involved in selective loss, but not formation, of neural connections. The role of COX-2 in human brain function and the potential impact of specific COX-2 inhibitors is unknown and requires evaluation, especially in view of the well-known negative impact of non-specific COX inhibitors on cognitive function in the elderly.\(^{87}\)

Cyclooxygenase-2 may also play a specific role in local inflammation in the brain. In this regard COX-2 can be up-regulated by specific stimuli in microglial cells, the tissue-specific macrophages that reside in the brain in a dormant condition until activated during host defense or tissue remodeling.\(^{88}\) Unlike other inflammatory cells, the microglial cell up-regulates COX-2 only in response to direct lipopolysaccharide exposure and not to cytokines, a rare event linked with direct bacterial infection of the brain. Thus, the microglial defense is usually not part of the systemic response to inflammation, but may play a critical role during brain infection.

**Ovarian and Uterine Function**

Although classically associated with parturition,\(^{89}\) prostaglandins and COX-2 have now been implicated as mediators of other stages of pregnancy, including ovulation and implantation. Studies with COX-2–null mice have documented reproductive failures at ovulation, fertilization, implantation, and decidualization,\(^{35}\) indicating the essential role of COX-2 at each of these stages.

When the induction of COX-2 was first observed immediately following the luteinizing hormone surge, research suggested that this COX isoform may play a role in normal physiologic events.\(^{90}\) This COX-2 induction accompanies normal oocyte development and seems to be necessary to produce the proteolytic enzymes that rupture the follicles.\(^{91}\) The

inductive trigger for COX-2 during ovulation may involve luteinizing hormone and follicle-stimulating hormone, transforming growth factor\(\alpha,\)\(^{92}\) or interleukin-1\(\beta,\)\(^{96}\) leading to increased COX-2 gene transcription.\(^{38}\) Inhibition of COX-2 by NSAIDs may explain the infertility secondary to delayed or blocked follicular rupture associated with their use.\(^{95,96}\)

Following fertilization in the mouse, COX-2 also plays a role in embryo implantation in the uterine myometrium. Whereas COX-1 and specific prostaglandin receptors apparently prepare the wall for interaction with the embryo,\(^{56,60}\) the COX-2 enzyme, leading to the production of prostacyclin, seems to be necessary for the implantation event itself.\(^{59,97}\)

**Thrombosis**

Maintenance of normal blood flow and the appropriate thrombogenic response to injury requires a delicate balance between the activities of platelet-produced thromboxane \(A_2\) and endothelial cell–derived prostacyclin (Table). After activation, platelets produce thromboxane \(A_2\) via the action of COX-1, the only COX isoform they contain.\(^{59,73,76}\) Thromboxane \(A_2\) plays an essential role in the aggregation of platelets. The release of eicosanoids by activated platelets is thought to provide a substrate and a stimulus for the production of prostacyclin by endothelial cells.\(^{77}\) Prostacyclin stimulates vasodilatation, thereby countering the vasoconstrictive action of thromboxane \(A_2.\)\(^{7}\) It has recently been shown that shear stress induces COX-2 expression in endothelial cells and that substantial amounts of eicosanoid production by endothelial cells results from the action of COX-2.\(^{98}\) In fact, recent studies have shown that excretion of 2,3-dinor 6-keto prostaglandin \(F_1\alpha,\) a metabolite of prostacyclin that is indicative of systemic prostacyclin production in humans, was significantly inhibited by the administration of a specific COX-2 inhibitor.\(^{73,99}\) These results indicate that a substantial proportion of systemic prostacyclin production derives from the action of COX-2. These data are all consistent with the conclusion that platelet thromboxane \(A_2\) production is uniquely regulated by the action of COX-1, whereas a substantial portion of endothelial cell–derived prostacyclin may be produced as a result of the action of COX-2. Since currently available NSAIDs inhibit both COX-1 and COX-2, a balanced impact on these prothrombotic and antithrombotic activities is expected. However, specific COX-2 inhibitors may limit the production of prostacyclin by endothelial cells while having no effect on the production of thromboxane \(A_2\) by platelets. The resulting imbalance may then favor platelet aggregation and vasoconstriction with a resulting increase in the tendency for vascular occlusion and tissue ischemia. The potential clinical impact of this imbalance has not been explored, but should be examined, especially in patients at risk for ischemic events.

**SUMMARY**

A full awareness of the physiologic and pathophysiologic roles of COX-1 and COX-2 continues to emerge. Although the initial paradigm that COX-1 was homeostatic and COX-2 was proinflammatory provides a general conceptual framework, more recent investigation has clearly indicated more complex roles for these isoforms in both health and disease.
As specific COX-2 inhibitors progress to market, attention should be given to potential adverse effects related to the kidney, gastrointestinal tract, bone, and brain as well as a potential negative impact on pregnancy and thrombogenic potential. On the other hand, new potential therapeutic targets for specific COX-2 inhibitors have emerged as a role for COX-2 in the development and progression of adenomatous polyposis and colon cancer. Moreover, the possibility that COX-2 may play a role in the progression of Alzheimer disease has also been suggested. Thus, developing information about the biologic function of COX-2 has presented the clinician with new challenges as well as new opportunities.

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