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Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities

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Inhibitors selective for prostaglandin G/H synthase-2 (PGHS-2) (known colloquially as COX-2) were designed to minimize gastrointestinal complications of traditional NSAIDs — adverse effects attributed to suppression of COX-1–derived PGE_2 and prostacyclin (PGI_2). Evidence from 2 randomized controlled-outcome trials (RCTs) of 2 structurally distinct selective inhibitors of COX-2 supports this hypothesis. However, 5 RCTs of 3 structurally distinct inhibitors also indicate that such compounds elevate the risk of myocardial infarction and stroke. The clinical information is biologically plausible, as it is compatible with evidence that inhibition of COX-2–derived PGI_2 removes a protective constraint on thrombogenesis, hypertension, and atherogenesis in vivo. However, the concept of simply tipping a "balance" between COX-2–derived PGI_2 and COX-1–derived platelet thromboxane is misplaced. Among the questions that remain to be addressed are the following: (a) whether this hazard extends to all or some of the traditional NSAIDs; (b) whether adjuvant therapies, such as low-dose aspirin, will mitigate the hazard and if so, at what cost; (c) whether COX-2 inhibitors result in cardiovascular risk transformation during chronic dosing; and (d) how we might identify individuals most likely to benefit or suffer from such drugs in the future.

One should not increase, beyond what is necessary, the number of entities required to explain anything.
— Occam's razor

Arachidonic acid (AA) is subject to metabolism by prostaglandin G/H synthase (PGHS; commonly known as COX) enzymes, lipoxygenases, and epoxygenases to form a mesmerizing array of biologically active products. The COX enzymes are bisfunctional proteins, possessing both COX and hydroperoxidase (HOX) activities, catalyzing the biotransformation of AA into the PG endoperoxide intermediates PGG_2 and PGH_2. These are, in turn, acted on by isomerases and synthases to form the PGs and thromboxane A_2 (TxA_2) (1–3). All of these products activate G protein–coupled receptors; the phenotypes resulting from deletion of these receptors has informed considerably our understanding of prostanoid biology (4). NSAIDs, which include both traditional NSAIDs (tNSAIDs) and selective inhibitors of COX-2 and which are among the most commonly used drugs (5), relieve pain and inflammation by suppressing the COX function of PGHS and the consequent formation of PGE_2 (6) and prostacyclin (PGI_2) (7), but perhaps also of other prostanoids. The failure of NSAIDs to inhibit PGHS HOX–dependent free radical formation may contribute to the failure of these drugs to modify disease progression in arthritis (8).

Several groups made observations (9–12) that predicted the discovery of a second COX enzyme (13–15). Unlike COX-1, which appeared to be expressed constitutively in most tissues, COX-2 was subject to rapid induction by inflammatory cytokines and mitogens and was speculated to account largely if not exclusively for PG formation in inflammation and cancer. Drug screening in cellular and biological systems identified compounds selective for COX-2 (16, 17). The elucidation of the COX structures subsequently explained this capability (18, 19). The COX enzymes are remarkably similar, both sharing a hydrophobic tunnel that affords access of the lipid substrate to the active site, deep within the proteins. However, the COX-2 tunnel is more accommodating and includes a side pocket not present in COX-1 (Figure 1). This affords both broader substrate recognition and a structural explanation for the ability to detect drugs selective for COX-2 in pharmacological screens (20). The attraction in developing such compounds was that they might not induce the commonest complication of tNSAIDs — gastrointestinal (GI) intolerance — which the "COX-2 hypothesis" attributed entirely to inhibition of COX-1–derived protective PGE_2 and PGI_2 by gastroduodenal epithelium and platelet COX-1–derived TxA_2 (21). Subsequently, the simplicity of this concept has been challenged by increasing evidence supporting the importance of COX-2 in resolution of mucosal inflammation and in ulcer healing (22). Despite this and epidemiological evidence suggesting that the incidence of severe tNSAID-related GI adverse effects was in decline (23), the race for the approval of drugs designed as COX-2–specific inhibitors gained momentum. The conventional bases for approval by the FDA of the first 3 of these drugs — celecoxib, rofecoxib, and valdecoxib —were relatively small: clinical studies consisted mostly of hundreds of volunteers and were short-term (mostly less than 6

Nonstandard abbreviations used: AA, arachidonic acid; APC, Adenoma Prevention with Celecoxib; APPROVe, Adenomatous Polyp Prevention on Vioxx; CABG, coronary artery bypass grafting; CLASS, Celecoxib Long-term Arthritis Safety Study; EDGE, Etoricoxib versus Diclofenac Sodium Gastrointestinal Evaluation; EMEA, European Medicines Agency; GI, gastrointestinal; HOX, hydroperoxidase; IP, PGI_2 receptor; MEDAL, Multinational Etoricoxib and Diclofenac Arthritis Long-term; PGHS, prostaglandin G/H synthase; PGI_2, prostacyclin; PGIM, PGI_2 metabolite; RCT, randomized controlled-outcome trial; TARGET, Therapeutic Arthritis Research and Gastrointestinal Event Trial; tNSAID, traditional NSAID; TxA_2, thromboxane A_2; VIGOR, Vioxx Gastrointestinal Outcome Research.

Conflict of interest: G.A. FitzGerald receives financial support for investigator-initiated research from Bayer, Merck, and Boehringer Ingelheim, all of which manufacture drugs that target COXs. G.A. FitzGerald is a member of the Steering Committee of the Multinational Etoricoxib and Diclofenac Arthritis Long-term (MEDAL) Study Program. This author also serves as a consultant for Johnson & Johnson, Bayer, Merck, GlaxoSmithKline, Novartis, Boehringer Ingelheim, and NiCox.

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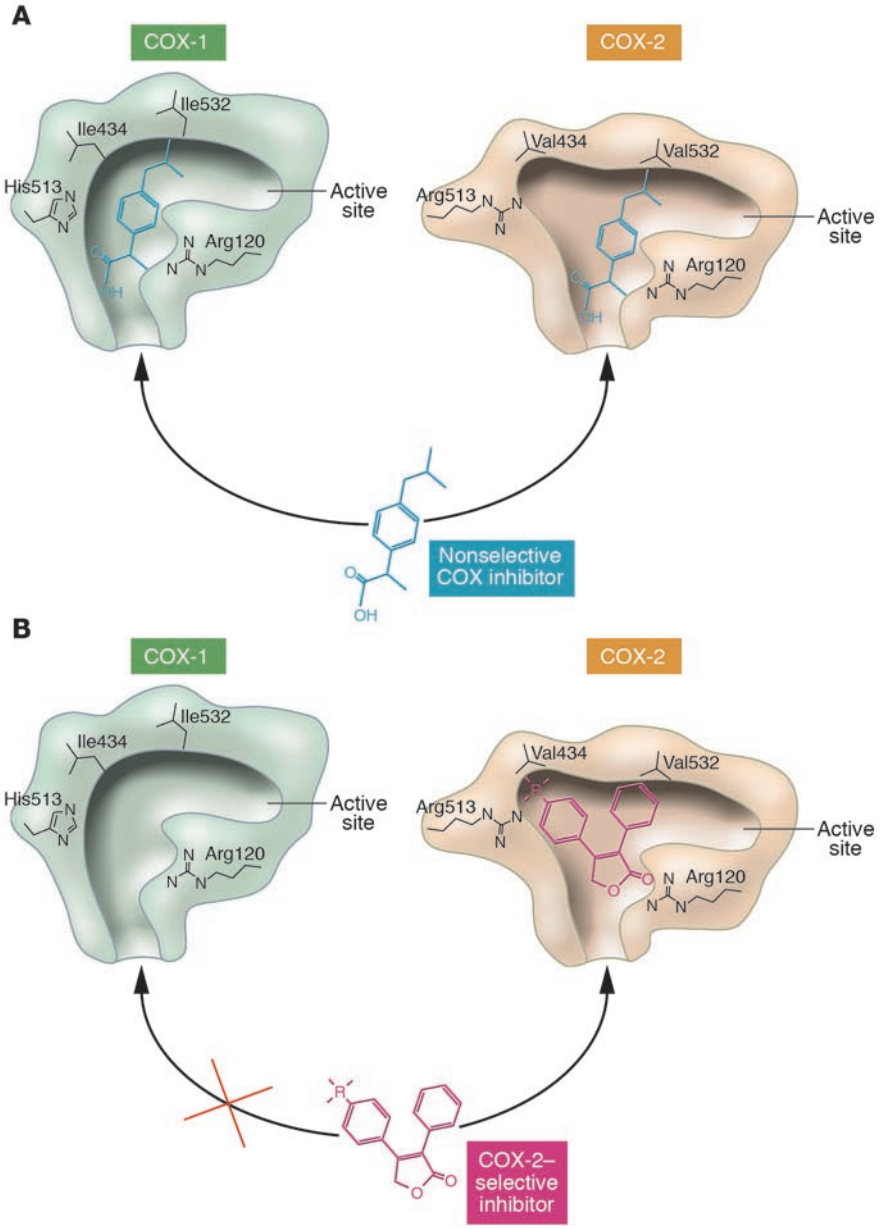

Figure 1
Schematic depiction of the structural differences between the substrate-binding channels of COX-1 and COX-2 that allowed the design of selective inhibitors. The amino acid residues Val434, Arg513, and Val523 form a side pocket in COX-2 that is absent in COX-1. (**A**) Nonselective inhibitors have access to the binding channels of both isoforms. (**B**) The more voluminous residues in COX-1, Ile434, His513, and Ile532, obstruct access of the bulky side chains of COX-2 inhibitors. Figure modified with permission from *Nature* from protein structures reported in refs. 18 and 20.

months) studies in which endoscopic visualization of drug-induced ulceration was compared among the coxib, a tNSAID, and placebo. The superiority of the COX-2 inhibitors over their tNSAID comparators in these studies was striking (24–28).

Mechanistic basis for a cardiovascular hazard resulting from inhibition of COX-2

During the course of drug development, we found that both celecoxib and rofecoxib suppressed urinary 2, 3-dinor 6-keto $PGF_{1\alpha}$, a stable PGI_2 metabolite (PGIM), to a degree comparable to that attained by treatment with structurally distinct tNSAIDs (29, 30). While the latter drugs inhibited platelet aggregation ex vivo transiently at the time of peak action, the coxibs had no such effect, compatible with the absence of COX-2 from mature human platelets (31). Unlike the tNSAID comparators in these studies — ibuprofen and indomethacin — neither celecoxib nor rofecoxib inhibited COX-1–derived TxA_2 coincident with its impact on PGI_2. Thus, the cardiovascular effects of TxA_2 would be expected to be exaggerated. However, as PGI_2 was known to act as a general restraint on *any* recognized stimulus to platelet activation, it was not suggested that upsetting a notional "balance" between the 2 prostanoids was likely to be the mechanism of drug action. Correspondingly, variation in other endogenous mediators, such as NO, would be expected to modulate the impact of COX-2 inhibition on cardiovascular function. Given that similar observations were made with both celecoxib and rofecoxib, it appeared that this effect was mechanism based, rather than an off-target effect restricted to 1 compound. Despite the presence of only COX-1 in endothelial cells under static conditions in vitro (32), PGI_2 — a dominant product of endothelium (33) — appeared largely to derive from COX-2 under physiological conditions in humans. Prior findings of Topper and Gimbrone (34) were invoked to explain these observations. They had found that subjection of endothelial cells in culture to laminar shear upregulated COX-2 expression. Thus, induction of COX-2 was likely to have occurred in response to blood flow under physiological conditions in vivo. Studies that led to approval of the coxibs were too short and too small in subject number to have excluded a risk of myocardial infarction or stroke attributable to this hypothesis.

Insight into the consequences of suppressing PGI_2 in vivo were limited at the time; deletion of the PGI_2 receptor (IP) augmented the response to an exogenous thrombogenic stimulus in mice (7). However, it was claimed that redundancy with other antithrombotic systems, particularly the elaboration of NO, would annul the impact of PGI_2 suppression in vivo. When deletion of the IP was shown to restrain the effect of endogenous TxA_2 on platelet activation and vascular proliferation in response to injury (35), it was questioned whether loss of 2 copies of the IP would mimic the substantial but incomplete suppression of PGIM attained in humans (S1). Subsequent experiments revealed that IP deletion predisposed to thrombosis in a gene dose–dependent fashion; the effect of a COX-2 inhibitor in thrombosis models was intermediate between $IP^{+/-}$ and $IP^{-/-}$ mice

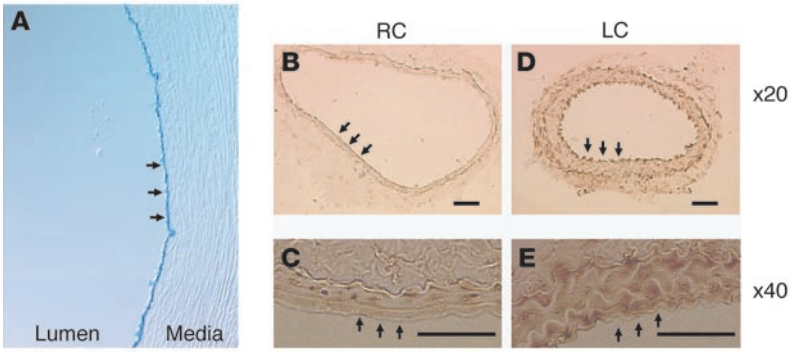

Figure 2
Expression of *COX-2* mRNA in the endothelium (arrows) of human (**A**) and COX-2 protein in murine (**B**–**E**) arteries. (**A**) In situ detection of *COX-2* mRNA in the endothelium of a human umbilical artery. Image kindly provided by James N. Topper, Frazier Healthcare Ventures, Palo Alto, California, USA. (**B**–**E**) Immunostaining shows COX-2 upregulation 4 weeks after left common carotid artery ligation in mice. Scale bars: 50 μm. Baseline COX-2 expression (brown staining) is evident in the intima in cross sections of the right common, unligated carotid artery, which served as a control. Magnification, ×20 (**B**); ×40 (**C**). Flow reduction induced further COX-2 expression in the intimal layer and marked endothelial expression as shown in **D** and **E**. Magnification, ×20 (**D**); ×40 (**E**). LC, left common carotid artery; RC, right common carotid artery. **B**–**E** are reproduced here with permission from *Circulation Research* (55).

(Y. Cheng, personal communication). It has also been suggested that the presence of PGIM does not reflect endothelial biosynthesis of PGI_2 and that COX-2 is undetectable in endothelial cells ex vivo (S2). While one can never attribute with certainty a tissue of origin to a metabolite measured in urine (36) or plasma (37), studies in vitro had indicated that endothelium is the major tissue source of PGI_2 (33) and local vascular stimulation and short-term systemic administration of PGI_2 is reflected by readily detectable alterations in urinary PGIM levels (38, 39). Furthermore, expression of *COX-2* is evident in human endothelial cells ex vivo (Figure 2A), and expression of endothelial COX-2 may be modulated in a flow-dependent manner in mice (Figure 2, B–F). Indeed, the human cDNAs for COX-2 were originally cloned from unstimulated endothelial cells (40, 41), reflective of constitutive expression of the enzyme. Aside from physiological conditions, one would expect that vascular stimulation by the products of platelet activation and by inflammatory cytokines might upregulate endothelial and vascular smooth muscle cell expression of COX-2, as occurs in atherosclerotic lesions (42, 43). Indeed, excretion of both PGIM and the TxA_2 metabolite 2, 3-dinor TxB_2, are together increased in patients with severe atherosclerosis (43, 44, S3). The failure of some studies to report COX-2 expression in endothelial cells ex vivo may reflect the particular experimental circumstances and/or discordance between the offset kinetics of flow-induced gene expression and the time of sample preparation.

Subsequent work expanded our understanding of the effects of PGI_2 on cardiovascular biology (Figure 3). Celecoxib suppressed PGI_2-dependent vascular bioactivity and undermined the antithrombotic effect of aspirin in dogs (45). Selective COX-2 inhibition suppressed PGI_2 and predisposed to platelet activation and arterial thrombosis under conditions of hypoxia-induced pulmonary hypertension in rodents (46). Similarly, selective COX-2 inhibition suppressed PGI_2 and enhanced platelet–vessel wall interactions in vivo and platelet adhesion to hamster cheek pouch arterioles (47). PGI_2 was shown in endothelial cells to stimulate substantially thrombomodulin (48). Thus, removal of this natural constraint to thrombin activation would interact with augmented platelet activation to promote assembly of the prothrombinase complex and consequent thrombosis, perhaps particularly in the microvasculature. Reperfusion injury of the myocardium is augmented in mice lacking the IP (49), suggesting a limit to the benefit of such therapeutic strategies in patients who suffered a thrombosis, and COX-2–dependent PGI_2 was shown to afford protection against oxidant injury to cardiomyocytes in vivo (50).

Aside from effects most relevant to acute human syndromes of thrombotic vascular occlusion, suppression of COX-2 may also predispose to a more gradual elevation of cardiovascular risk during prolonged dosing with inhibitors. Deletion of the IP was found to promote initiation and early progression of atherosclerosis in mice genetically predisposed to hyperlipidemia (51, 52). This appears to reflect removal of a constraint to the activation of both neutrophils and platelets, their interaction with the vessel wall, and the resultant oxidant stress. Additionally, COX-2–dependent PGI_2 formation appears to contribute to the atheroprotection afforded by estrogen and mediated via its ER-α receptor in vivo (52). Deletion of the IP (53, 54), like COX-2 inhibition (37), elevates blood pressure (54) and augments the pressor response to dietary sodium (53, 54). Finally, both deletion of the IP and inhibition of COX-2 modulate vascular remodeling induced by hemodynamic stress — such as hypertension — in vivo (55). Additional effects on AA metabolism resulting from COX-2 inhibition, including a failure to metabolize the vasoconstrictor 20-hydroxyeicosatetraenoic acid (20-HETE) to a vasodilator product (56) and augmented metabolism via lipoxygenase and cytochrome P450 enzymes, may impact blood pressure regulation. However, evidence that such effects contribute to the renovascular effects of COX-2 inhibitors remains to be provided in vivo. COX-2 is also the dominant source of PGE_2 and PGD_2 biosynthesis under physiological conditions in humans (S4, S5). Deletion of the EP2 receptor for PGE_2, like deletion of the IP, results in salt-sensitive hypertension (57), and both PGE_2 (via the IP) and PGD_2 inhibit platelet activation, at least in vitro (S6, S7). The extent to which inhibition of these other PGs might contribute to a cardiovascular hazard of COX-2 inhibitors remains to be established. In the interim, one might speculate how the disparate effects of PGI_2 suppression on atherogenesis, blood pressure, and the remodeling response might converge, over time, to result in transformation of cardiovascular risk in patients initially at low risk when exposed to chronic COX-2 inhibition (58).

In summary, a substantial body of evidence has accumulated that 1 mechanism, suppression of COX-2–dependent PGI_2 formation, can both augment the response to thrombotic and hypertensive stimuli and initiate and accelerate atherogenesis.

Concordance of clinical experience with mechanism-based predictions

Given the assumption that this mechanism explains the observed cardiovascular complications of COX-2 inhibitors, how would such a hazard be expected to become clinically manifest?

First, the actual degree of selectivity attained at the vascular interface in vivo would be an important variable. Although assays in whole blood in vitro suggest a clear segregation between the degree of selectivity attained by the drugs under consideration, there are

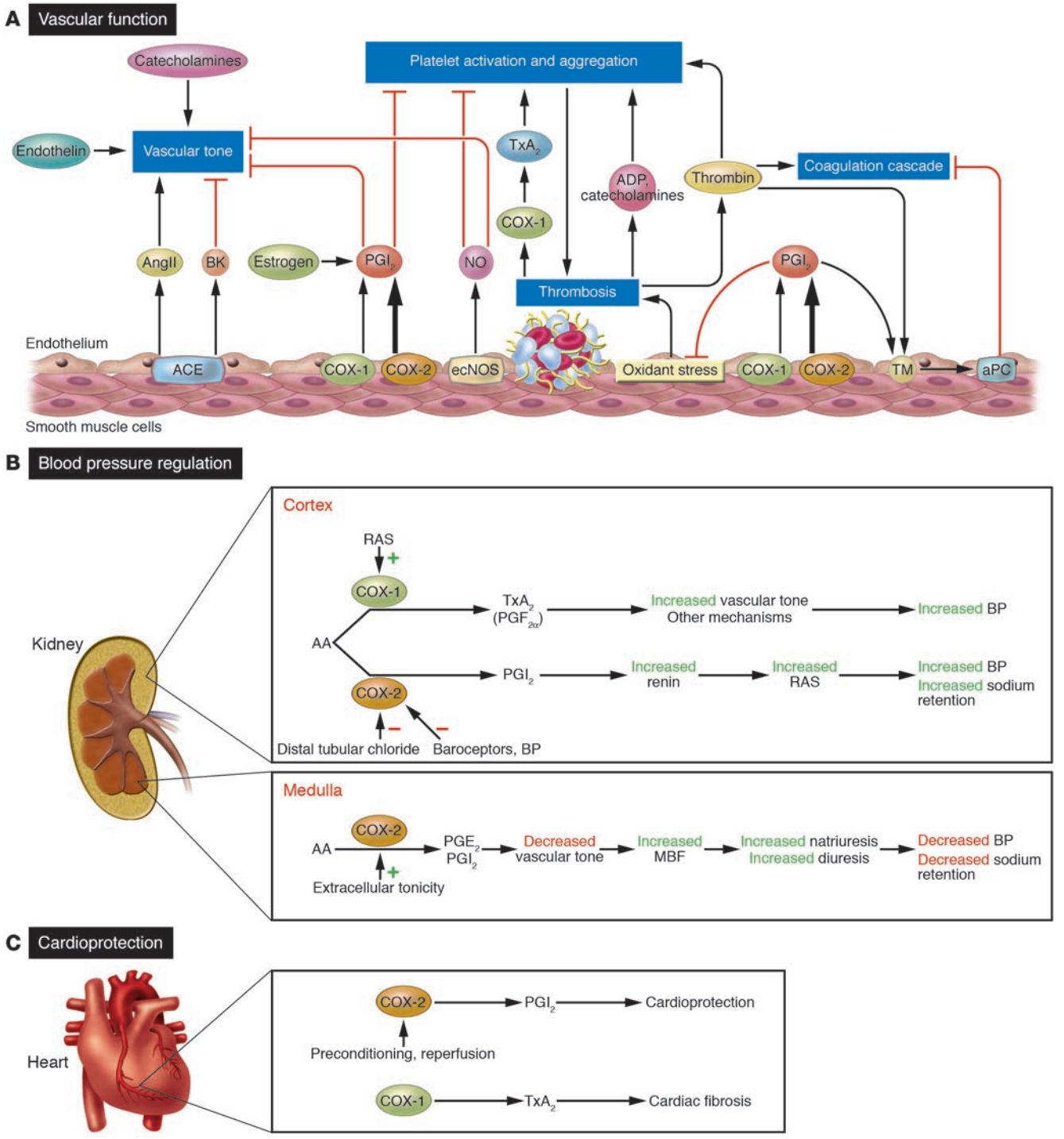

Figure 3
Roles of the COX isozymes in cardiovascular (**A** and **C**) and renal (**B**) biology. ACE, angiotensin-converting enzyme; ADP, adenosine diphosphate; aPC, activated protein C; BK, bradykinin; ecNOS, endothelial cell NOS; MBF, medullary blood flow; RAS, renin-angiotensin system; TM, thrombomodulin.

substantial interindividual differences in drug response (59) and consequent overlap in the degree of selectivity attained in vivo. Selectivity for COX-2 can be viewed as a continuous variable within the class of NSAIDs. Indeed, some tNSAIDs — diclofenac, nimesulide, meloxicam, and nabumetone — express average selectivity for COX-2 similar to that of celecoxib in human whole blood in vitro (28). Sufficient concentration of any selective COX-2 inhibitor becomes nonselective as it begins to inhibit COX-1, at least in vitro (28, 60). Second, the more prolonged the drug exposure (determined by dose, duration of action, and duration of treatment), the more likely an

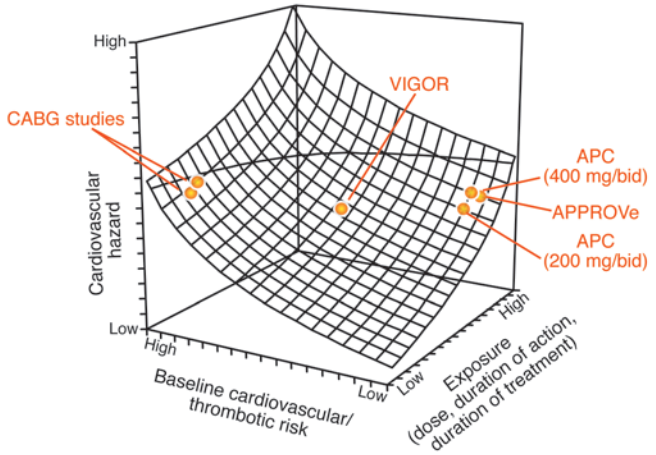

Figure 4
Illustration of the expected interaction of baseline cardiovascular and thrombotic risk with components of drug exposure including dose, duration of action, and duration of treatment with a selective inhibitor of COX-2. The approximate relationship of cardiovascular hazard detected in controlled studies within this interaction are indicated (not to scale). APC study, ref. 81; APPROVe study, ref. 72; CABG studies, parecoxib/valdecoxib after bypass surgery, refs. 77, 78; VIGOR study, ref. 62.

adverse consequence. Third, concordant administration of low-dose aspirin, which favors inhibition of COX-1 (61), would be expected to mitigate but not abolish the hazard. The degree and duration of simultaneous inhibition of the 2 COX enzymes would also be expected to influence the existence of a cardiovascular hazard from tNSAIDs (see below). Finally, $IP^{-/-}$ mice are more responsive to thrombogenic stimuli; they do not develop spontaneous thrombosis (7). Thus, a clinical or genetic predisposition to thrombosis would favor emergence of a drug-related cardiovascular event.

Aside from the question posed by clinical pharmacology, the first evidence consistent with the hypothetical cardiovascular hazard emerged in the Vioxx Gastrointestinal Outcome Research (VIGOR) study (62), in which a 2-fold divergence in the incidence of serious GI adverse events between rofecoxib and the tNSAID naproxen coincided with a 5-fold divergence in the incidence of myocardial infarction (20 versus 4 events). This study was conducted with a high dose (50 mg/day) of rofecoxib in patients in whom low-dose aspirin was precluded. Most of the patients suffered from RA, a disease associated with an odds ratio of a myocardial infarction roughly 50% higher than in patients with osteoarthritis or no arthritis (63). These results generated considerable controversy; some researchers claimed that rofecoxib was neutral and that the result reflected a cardioprotective effect of naproxen, based on its extended duration of action (64), permitting this mixed inhibitor of COX-1 and COX-2 to behave like aspirin.

The corresponding outcomes study of celecoxib (Celecoxib Long-term Arthritis Safety Study [CLASS]) was published in a highly unorthodox manner (65). Partial presentation of the data seemed to suggest that high-dose (800 mg/day) celecoxib had caused fewer GI adverse effects than its tNSAID comparators; however, this turned out not to be the case when the full data set was revealed (66). This study, conducted with, on average, a shorter-lived, less selective COX-2 inhibitor than rofecoxib, also demonstrated no difference in the incidence of cardiovascular events. Around 20% of the patients took aspirin, and much was made of the apparent divergent incidence of GI adverse effects on ibuprofen versus celecoxib in a post hoc analysis of nonaspirin users. Perhaps aspirin had masked the GI advantage of celecoxib. However, if so, it may also have masked the cardiovascular hazard. A similar underpowered and retrospective analysis suggests that cardiovascular events occurred more often with celecoxib than with ibuprofen in nonaspirin users. Interestingly, the incidence of both GI and cardiovascular events on diclofenac and celecoxib appeared to be similar (67).

In summary, the number of events reported in the VIGOR study was small. However, if the estimate of the difference between the 2 treatment groups was reliable, this was larger than might be expected from an "aspirin-like" effect of naproxen; clearly it was

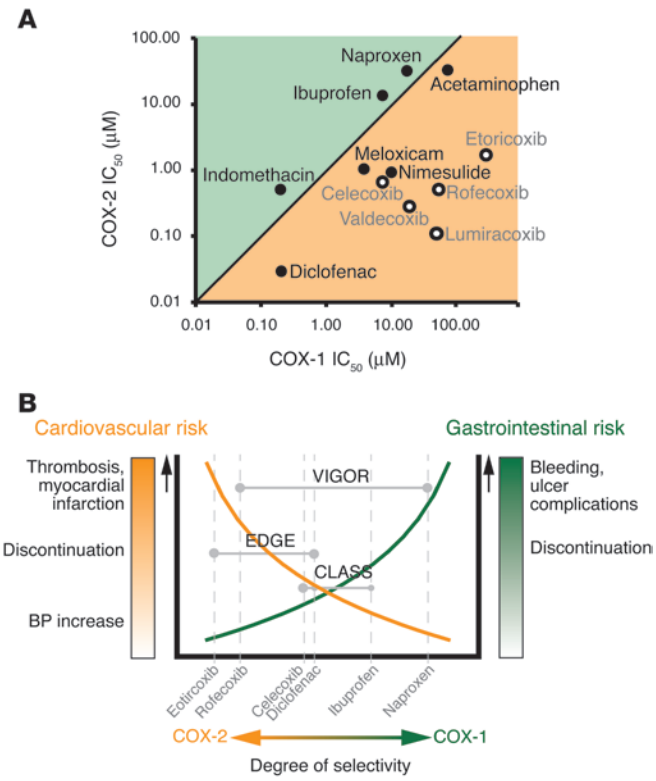

Figure 5
The spectrum of selectivity for COX inhibition. (**A**) The relative affinities of tNSAIDs and coxibs (open circles) for COX-1 and COX-2. The concentrations required to inhibit COX-1 and COX-2 by 50% (IC_{50}) have been measured using whole-blood assays of COX-1 and COX-2 activity in vitro. The diagonal line indicates equivalent COX-1 and COX-2 inhibition. Drugs plotted below the line (orange) are more potent inhibitors of COX-2 than drugs plotted above the line (green). The distance to the line is a measure of selectivity. Note the log scale. For example, lumiracoxib is the compound with the highest degree of selectivity for COX-2 as its distance to the line is the largest. Celecoxib and diclofenac have similar degrees of selectivity for COX-2, as their distances to the line are similar; however, diclofenac is active at lower concentrations and thus located more to the left. Figure modified with permission from *The New England Journal of Medicine* (28). (**B**) Implication of the relative degrees of selectivity. Increasing degrees of selectivity for COX-2 are associated with augmented cardiovascular risk while increasing degrees of selectivity for COX-1 are associated with augmented GI risk. The relative size of the circles indicates approximately the variation in sample sizes among the trials.

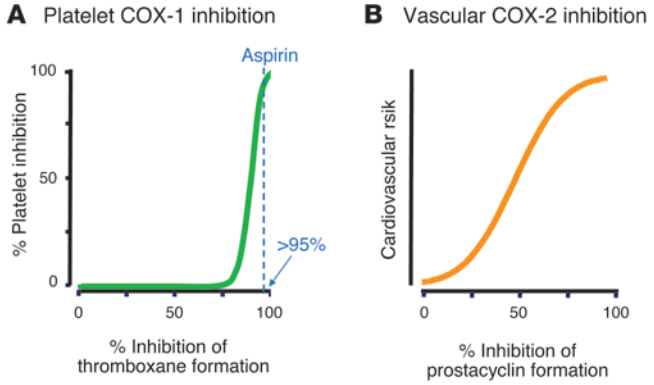

Figure 6
Discordant dose-response relationships for inhibition of platelet COX-1 (**A**) and vascular COX-2 (**B**). Derived from data reported in ref. 28.

compatible with the coincidence of a cardiovascular hazard from rofecoxib and some protection from naproxen.

The traditional approach to drug safety is to rely upon pharmacoepidemiology. However, this is an insensitive detector system when the need is to identify a small absolute increase — maybe 1–2% in retrospect — in the absolute incidence of a problem that occurs commonly in the age group under study. In addition to these limitations, epidemiological (observational) studies are subject to many sources of bias, and in this particular case, the common use of prescription databases was also potentially confounded by unrecorded over-the-counter use of tNSAIDs and aspirin.

Further attention to the prospect of a cardiovascular hazard from the COX-2 inhibitors (coxibs) was prompted initially by a comparison of trial data for both drugs, including the VIGOR and CLASS studies, with a control group, based on data drawn from the placebo groups of 4 primary prevention trials of low-dose aspirin (68). However, this indirect analysis was subject to considerable methodological criticism. The estimated cardiovascular event rates in 2 of the placebo groups lay below while 2 were above those calculated for rofecoxib and celecoxib. Roughly 70% of the data for the pooled estimate in the control group was drawn from the first 2 studies. However, the controversy around this paper prompted a spate of observational studies. Several (69–71) but not all of these detected a cardiovascular hazard associated with 50 mg/d rofecoxib, but most failed to do so with lower doses such as that (25 mg/d) used in the randomized controlled-outcome trial (RCT), the Adenomatous Polyp Prevention on Vioxx (APPROVe) study, that subsequently led to the withdrawal of the drug (72). Most observational studies and overview analyses of the small, short studies that provided the basis for drug approval also failed to detect a hazard from celecoxib (73) and valdecoxib (74). Pharmacoepidemiology alone did not clearly discriminate between a hazard peculiar to rofecoxib and a mechanism-based effect.

The situation was clarified by the emergence of information from 4 published (and 1 still unpublished; ref. 75) placebo-controlled trials. The pattern of the clinical information was consistent with the proposed mechanism. For reasons discussed above, a prothrombotic clinical substrate would favor the rapid emergence of adverse cardiovascular events in a relatively small study. An example of such a setting is coronary artery bypass grafting (CABG), which is characterized by intense hemostatic activation (76). Two placebo-controlled studies of valdecoxib (77, 78), anteceded by its intravenous prodrug parecoxib, were performed in patients undergoing CABG. Despite their small study sizes (462 and 1636 patients, respectively) and short duration (10 and 14 days of treatment, respectively), pooled analysis of the 2 quite similar studies suggests that parecoxib/valdecoxib elevate the combined incidence of myocardial infarction and stroke by 3-fold in this population (79). Although the patients were prescribed aspirin, the timing of its administration relative to the incidence of the vascular events is unclear. CABG is also a setting of apparent "aspirin resistance" (80). These studies are compatible with the rapid emergence of a cardiovascular hazard based on suppression of COX-2–derived PGI_2 in a population with preexisting, intense hemostatic activation. Similarly, one would anticipate that a less pronounced prothrombotic substrate, such as the patients with RA in the 9-month VIGOR trial, might reveal a hazard more gradually. The rapidity with which a cardiovascular risk might become manifest would reflect in part the intensity of a genetic or environmental predisposition to thrombosis (Figure 4).

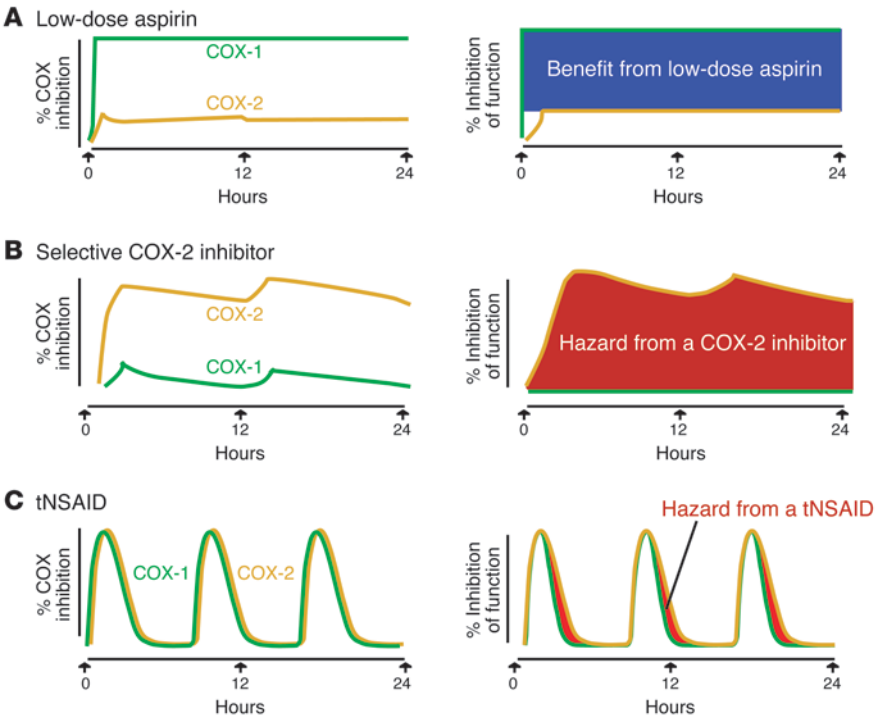

Figure 7
Clinical implications of differences in the dose-response relationships for COX-1 and COX-2 of low-dose aspirin (**A**), a selective inhibitor of COX-2 (**B**), and a tNSAID (**C**). The area between the dose-response curves would correspond to benefit (**A**) and hazard (**B** and **C**) and to the size of these effects.

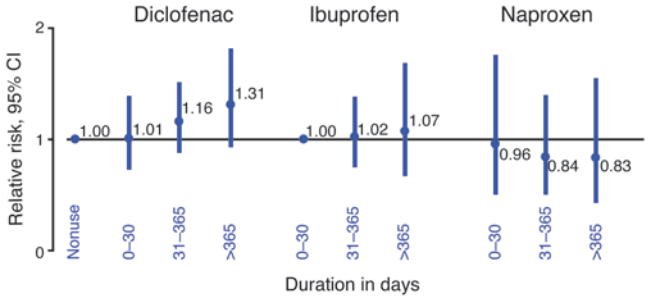

Figure 8
Duration of use of tNSAIDs and individual tNSAIDs among current users (use within a month) and risk of myocardial infarction. Redrawn with permission from *BMC Medicine* (106). CI, confidence interval; nonuse, reference group with relative risk of 1.00.

APPROVe (72) and Adenoma Prevention with Celecoxib (APC), 2 studies in patients with colonic adenomata, presumed initially to be at low risk of cardiovascular events, revealed the gradual emergence of a cardiovascular risk attributable respectively to rofecoxib (72) and celecoxib (81) after dosing for more than 1 year (Figure 4). Supportive of this being a true drug-related effect, the hazard in patients taking celecoxib 200 mg/bid and 400 mg/bid appeared to be dose related (81).

Several comparative studies of COX-2 inhibitors and tNSAIDs failed to detect a discriminant incidence of cardiovascular events. However, in each case, these studies were substantially underpowered to exclude this possibility. These include the Therapeutic Arthritis Research and Gastrointestinal Event Trial (TARGET) (82), which comprised two 1-year long, comparator studies of lumiracoxib, 1 with ibuprofen, 1 with naproxen. While cardiovascular events tended to be higher in the lumiracoxib group, the study included patients mostly at low risk, and the power of the comparisons was undermined (83). Furthermore, TARGET was not designed to establish noninferiority of cardiovascular risk among the treatment groups; thus, it had no predefined upper confidence interval for relative risk (84) and used an intention-to-treat analysis. While it has been suggested that the pharmacokinetics of lumiracoxib favor a transient exposure in the vascular compartment with prolonged availability in the joint space (85), 400 mg/d of lumiracoxib exceeds considerably the dose necessary to inhibit COX-2 at the time of peak drug action. Given at this dose, it has a prolonged systemic pharmacodynamic half-life, depressing PGIM excretion to a similar extent and for a similar duration as rofecoxib (ref. 86 and P. Patrignani, personal communication). Unpublished studies also substantially underpowered to exclude a cardiovascular hazard include the prematurely and unconventionally terminated Alzheimer's Disease Anti-inflammatory Prevention (ADAPT) study of celecoxib, naproxen, and placebo in Alzheimer disease and the multinational, placebo-controlled evaluation of celecoxib (400 mg/d) in chemoprevention of colonic adenomata (http://www.clinicaltrials.gov/ct/show/NCT00087256).

In summary, while the number of cardiovascular events in *all* of the relevant individual RCTs addressing this issue is small, the currently available clinical evidence is remarkably compatible with a unitary mechanism for which there is comprehensive biological plausibility, attained in vivo. The clinically concordant evidence includes the following: (a) the easiest detection of a signal in epidemiological studies for a long-lived compound with a high degree of selectivity for COX-2, rofecoxib, given at a high dose (50 mg/d); (b) the rapid emergence of a signal in 2 relatively small RCTs of valdecoxib in a setting of intense hemostatic activation and likely aspirin resistance; (c) the intermediate time to detection of a hazard in RA patients in the VIGOR study in whom hemostatic activation and risk of thrombosis is considerably less than in those individuals that have undergone CABG but exceeds that in patients without arthritis; (d) the similarity of the overview analyses of etoricoxib versus naproxen to what was observed in VIGOR (67) and evidence in trials to date (87) consistent with a cardiovascular hazard from this drug; (e) the delayed emergence of a hazard in 2 RCTs of prolonged treatment with rofecoxib and celecoxib, which is compatible with risk transformation in patients initially at low risk of cardiovascular disease; and (f) the evidence of hazard involving 3 structurally distinct selective COX-2 inhibitors — belying the notion that this is an off-target effect of rofecoxib. Finally, the issue of a mitigating effect of low-dose aspirin has not been addressed in the RCTs. This seems biologically plausible, as COX-1 knockdown in mice, which genetically mimics the impact of low-dose aspirin (88), attenuates the prothrombotic and hypertensive effect of COX-2 inhibition (Y. Cheng, personal communication). However, aspirin use was only prespecified in one of the RCTs in humans: TARGET. As mentioned, this was underpowered to address the cardiovascular question. However, the available evidence is compatible with risk attenuation; the relative risk of myocardial infarction was reduced from 2.37 in nonusers to 1.36 in those patients taking aspirin when lumiracoxib was compared with naproxen (83). However, this might also reflect a differential capacity of naproxen versus lumiracoxib to interact with and undermine the antiplatelet effect of low-dose aspirin (see below).

Cardiovascular risk and the tNSAIDs

Given the worldwide withdrawal of rofecoxib, the withdrawal of valdecoxib from the US, Australian, and European markets, and the substantial decline in the number of prescriptions for celecoxib, the safety of tNSAIDs has attracted substantial attention. Unfortunately, we do not have placebo-controlled RCTs addressing the cardiovascular safety of tNSAIDs, only observational studies, information from basic and human pharmacology, and the previously discussed tNSAID comparator RCTs. Aside from its relevance to clinical decision making, variability among the tNSAID comparators may be relevant to the heterogeneity of outcome among RCTs of COX-2–selective drugs.

A discussion of this information must be tempered by the reminder that all of these drugs, including those designed to be selective for COX-2, are NSAIDs and that the degree of selectivity among NSAIDs is best viewed as a continuous variable, given the substantial interindividual differences in response to drug administration (59). Thus, while valdecoxib is more selective for COX-2 than celecoxib in vitro, each individual will have idiosyncratic factors that modulate his or her dose-response relationship; there may well be some patients in whom celecoxib is the more selective inhibitor in vivo. It became fashionable after the VIGOR study to compare naproxen with "nonnaproxen" tNSAIDs. This approach should be abandoned, given the likely heterogeneity among the latter group with respect to cardiovascular risk.

Several tNSAIDs resemble, even in vitro, the selectivity profile of celecoxib (Figure 5A). These include diclofenac, the most commonly consumed tNSAID worldwide, and meloxicam, a marked beneficiary of the recent shift in NSAID prescriptions since the withdrawal of rofecoxib and valdecoxib in the US. Besides this pattern of selectivity, additional data substantiate the likelihood that diclofenac resembles

Table 1
Suggested consideration for preferred treatment options (level of evidence)[A]

COX inhibitors with proven cardioprotective efficacy	Low-dose aspirin (1a)
COX inhibitors with potential cardioprotective efficacy, variable among individuals	Naproxen (3a) Ibuprofen (3a)
COX inhibitors with the potential to offset the cardioprotective effect of low-dose aspirin	Flubiprofen (5) Indomethacin (5) Naproxen (5)
COX -2 inhibitors with proven gastroprotective efficacy	Rofecoxib (withdrawn) (1b) Lumiracoxib (FDA approval pending) (1b)
Treatment options for chronic treatment of patients with low cardiovascular and low GI risk	Naproxen (2b, 2a) Ibuprofen (2b, 2a)
Treatment options for chronic treatment of patients with low cardiovascular and high GI risk	Naproxen + proton pump inhibitor (2b, 2a) Ibuprofen + proton pump inhibitor (2b, 2a) Diclofenac + proton pump inhibitor (2b, 2a) Possibly celecoxib (although GI advantage vs. tNSAID not proven) (3, 2)
Treatment options for chronic treatment of patients with high cardiovascular and low GI risk	Naproxen + Clopidogrel (to avoid potential interaction with low-dose aspirin; however, the GI toxicity of this combination is likely to be at least that of tNSAID + low-dose aspirin and may warrant addition of a proton pump inhibitor) (5) Ibuprofen + clopidogrel (see comment above) (5)
Treatment options for chronic treatment of patients with high cardiovascular and high GI risk	Naproxen + proton pump inhibitor + clopidogrel (5) Ibuprofen + proton pump inhibitor + clopidogrel (5)

[A]The levels of evidence are based on the scoring system of the Oxford Centre for Evidence-Based Medicine (http://www.cebm.net/levels_of_evidence.asp): 1a, systematic reviews (with homogeneity) of RCTs; 1b, individual RCTs (with narrow confidence interval); 1c, all or none RCTs; 2a, systematic reviews (with homogeneity) of cohort studies; 2b, individual cohort study or low-quality RCTs; 2c, outcomes research, ecological studies; 3a, systematic review (with homogeneity) of case-control studies; 3b, individual case-control study; 4, case series (and poor quality cohort and case-control studies); 5, expert opinion based on physiology, bench research, or first principles.

celecoxib in practice. Neither cardiovascular nor GI outcomes differed between high-dose diclofenac and celecoxib in the CLASS study, and this pattern was sustained in the post hoc analysis in nonusers of aspirin. Secondly, NSAIDs that inhibit COX-1, such as ibuprofen, may interact pharmacodynamically to undermine the cardioprotective effects of low-dose aspirin (89). This interaction does not occur with drugs selective for COX-2, as it is not extant in platelets. Diclofenac, just like rofecoxib and celecoxib, but unlike ibuprofen or naproxen, does not subserve this interaction (89–91). Interestingly, a small epidemiological study of survivors of myocardial infarction suggested that concurrent ibuprofen but not diclofenac undermined the efficacy of aspirin in preventing a second myocardial infarction (92). It is unknown whether differences in the degree of selectivity attained on average by diclofenac versus purpose built COX-2 inhibitors will translate into differences in clinical outcomes. However, the Multinational Etoricoxib and Diclofenac Arthritis Long-term (MEDAL) study program (93, 94) consists of 3 randomized, double-blind trials in osteoarthritis and RA patients comparing etoricoxib to diclofenac (150 mg/d). Roughly 35,000 patients will be randomized, and the primary analysis is a noninferiority comparison of confirmed cardiovascular events, defined as an upper bound of the 95% confidence interval less than 1.30 (93, 94). The MEDAL program incorporates 2 smaller studies, the Etoricoxib versus Diclofenac Sodium Gastrointestinal Evaluation (EDGE) trials, designed primarily to evaluate GI outcomes respectively in patients with osteoarthritis (EDGE) and RA (EDGE 2). Etoricoxib was used at 90 mg/d in both EDGE studies but at 60 mg/d in the later stages of the MEDAL program. Interestingly, while GI and cardiovascular outcomes did not differ between the treatment groups in EDGE, discontinuations due to GI intolerance were more common with diclofenac while discontinuations due to hypertension were more common on etoricoxib (93, 94). Assimilation of the information from these comparator trials may begin to define the functional implications of progressive selectivity for COX-2 among the NSAIDs (Figure 5B). Less information is available concerning the other tNSAIDs with selectivity similar to diclofenac. Overview analyses suggest that serious GI toxicity is dose related with meloxicam, and at 15 mg/d this was similar to diclofenac. However, this impression was based largely on studies of fewer than 60 days duration (95), and there have been no adequately sized RCTs to address the GI toxicity of this compound (96). Similarly, we have no outcome data on cardiovascular events caused by meloxicam. Even less information is available with respect to nimesulide (97) and nabumetone (98).

A second group of tNSAIDs includes ibuprofen, flubiprofen, and indomethacin. These drugs favor somewhat inhibition of COX-1 over COX-2 in vitro and inhibit both enzymes reversibly during the dosing interval. Pharmacodynamic studies have raised the possibility that such drugs interact with and undermine the cardioprotective effect of aspirin (89). While observational studies support (92, 99, 100) and fail to support (101, 102) this hypothesis, the issue has not been addressed in an adequately powered RCT. Provocatively, concurrent high-dose ibuprofen appeared to undermine the benefit of aspirin in the TARGET trial (82); however, the number of events was too small to address the issue with confidence.

Most observational studies of tNSAID use last less than 1 year and most (102) but not all (103, 104) are consistent with no increased risk of cardiovascular events on ibuprofen. However, it is theoretically possible that a cardiovascular hazard from ibuprofen, albeit

considerably less pronounced than for selective inhibitors of COX-2, may exist. First, this may derive from discordant rates of offset of inhibition of the 2 COX enzymes in vivo. This has never been documented, but should inhibition of COX-1 wane faster than that of COX-2, the drug would be selective for the latter enzyme for some portion of the dosing interval. A second possibility pertains even if the inhibition time profiles of the 2 enzymes are dynamically aligned during the dosing interval. We had previously shown a highly nonlinear relationship in humans between the degree of inhibition of platelet COX-1 and platelet TxA_2–dependent function (105); one must inhibit the capacity of the enzyme by greater than 95% before impacting on platelet activation in vivo (Figure 6A). Studies in mice, which reveal that deletion of the IP has a gene dose–dependent effect on thrombosis and vascular function (Y. Cheng, personal communication), are consistent with a more linear relationship between inhibition of COX-2 and the functional consequences of suppressing COX-2–derived PGI_2 formation (Figure 6B). Discordance between these 2 curves might result in a "window of hazard," despite dynamic alignment of enzyme inhibition during the dosing interval (Figure 7A). Should such a window exist, it would be a smaller aperture than that for the risk from sustained selective inhibition of COX-2 throughout the dosing interval or, indeed, for the sustained benefit from inhibiting platelet COX-1 by low-dose aspirin (Figure 7, B and C). Thus, one would have to conduct much larger and/or longer trials to detect a hazard from ibuprofen than was necessary to detect a cardiovascular hazard from the coxibs or a benefit from low-dose aspirin. Interestingly, a recent epidemiological analysis of long-term use of NSAIDs suggests heterogeneity of effect consistent with their pharmacology (106). There is an apparent time-dependent emergence of a hazard with diclofenac; the data are also consistent with the possibility of a small hazard from ibuprofen only emerging upon prolonged dosing, if at all, while naproxen (see below) seems neutral or somewhat protective (Figure 8).

Naproxen has attracted particular attention because of the outcome of the VIGOR trial (62) and evidence of a prolonged pharmacokinetic half-life, at least in some individuals (64, 107). It is assumed that drugs that act like ibuprofen do not afford cardioprotection because they act reversibly and only sustain inhibition of platelet COX-1 in the functionally relevant zone transiently in the dosing interval (89). Epidemiological analyses of naproxen have suggested that it might have a dilute aspirin effect (103) consistent with an extended half-life and sustained platelet inhibition in some but not all individuals (64, 107). Indeed, this benefit of naproxen might be further undermined by irregular compliance outside the rigors of an RCT. We do not have evidence from cardiovascular outcome studies for naproxen and can only speculate as to how it may have contributed to the result reported in the VIGOR study. Recently, naproxen was shown to interact with aspirin in a manner similar to ibuprofen (91).

Aside from their putative effects on thrombosis, the potential of all NSAIDs, including those selective for COX-2, to raise blood pressure is well recognized. This may reflect diverse effects on salt and water handling and vascular reactivity, which have been discussed in detail elsewhere (108). The propensity of COX-2 deletion to elevate blood pressure is dependent on genetic background in mice (109), and it seems likely that genetic modifiers condition the existence and magnitude of this sporadic response to NSAID intake in humans. Despite the suggestion from experiments in mice (110) that hypertension might result most commonly from inhibition of COX-2 and the selectivity with which that is achieved, this has never been addressed directly by studies in humans, although an overview analysis consistent with that hypothesis has been published recently (111). Hypertension, reported as a serious adverse event, related to dose with rofecoxib and celecoxib and was more common with the more selective and longer-lived drug. Other factors, such as a documented COX-independent effect on vascular function (112) and drug potency, may explain perceived frequency of hypertension in patients taking indomethacin. Given COX inhibition by acetaminophen, reports of hypertension on this drug (113) are unsurprising. The commonest daily dose, 1000 mg, results in approximately 50% inhibition of both COX-1 and COX-2 (89), and an observational study suggests that higher doses, which may attain complete inhibition, result in a GI adverse event profile as in the tNSAIDs (114). It is unknown whether other aspects of acetaminophen action may modulate the impact of COX inhibition on cardiovascular function.

In summary, we lack information from placebo-controlled RCTs on the cardiovascular effects of the tNSAIDs. Thus, while a small but absolute risk of cardiovascular events is established for rofecoxib, valdecoxib, and celecoxib, we have no evidence of comparable quality for the tNSAIDs. Presently, it seems plausible to think of them in several clusters: (a) drugs such as diclofenac and meloxicam that are likely to resemble celecoxib with a small, but absolute risk; (b) drugs such as ibuprofen which, in themselves, may be neutral but may undermine the effectiveness of aspirin; (c) naproxen, which may afford protection in some individuals but which may also interact with aspirin; and finally, (d) a heterogeneous group of drugs such as indomethacin and acetaminophen, which may possess off-target cardiovascular effects that compound their profile. Clearly, the assumptions that underlie this classification can only be tested by RCTs. In the interim, however, it may afford a reasonable basis for therapeutic decision making (Table 1).

Some lessons learned and outstanding questions

An ambivalent legacy surrounds the aggressive strategy, heavily reliant upon direct-to-consumer marketing, that rendered the selective COX-2 inhibitors "blockbuster drugs." Ironically, the rationale for their development supported a niche concept — patients who had GI intolerance for tNSAIDs. After the drug withdrawals, it has been estimated that less than 5% of the patients previously taking coxibs had been at high risk of serious GI adverse effects from tNSAIDs (115).

An interdisciplinary approach to drug surveillance. The questions raised by mechanistic studies in humans performed before the first selective inhibitors of COX-2 were approved failed to prompt further studies to address the hypothetical mechanism of a cardiovascular hazard by the manufacturers. When such proof of concept did emerge, the data failed to inform substantially the interpretation of the pharmacoepidemiology. However, these different silos of information, mechanistic studies in humans, proof-of-principal studies in mice, and observational studies, finally afforded a powerful context within which to interpret the RCTs. In the future, we need to develop a more integrated, translational approach to information on drug safety, continuously refining our perception, perhaps exploiting formal Bayesian decision-making strategies as applied commonly in other fields. Postmarketing surveillance or pharmacovigilance might be strengthened considerably by the integration of large-scale databases from third-party payers, provisional periods of drug approval, and access to individual data from industry-sponsored clinical trials for independent analysis. However, such developments must be integrated within a surveillance system that prompts

rapid performance of mechanistic studies to address hypotheses of concern, even when they emerge after drug approval.

An individualized approach to defining efficacy and risk. Even though the development strategy of the coxibs largely bypassed their comparative efficacy with tNSAIDs, it is often claimed that these drugs work uniquely in some patients; however, the evidence is strictly anecdotal. It is possible to design studies to determine if there is variability between individuals in the efficacy of NSAIDs and, if so, to provide an explanation. The pharmacological response to administration of distinct selective COX-2 inhibitors is strikingly variable among individuals, in part due to genetic sources of variance (59); factors such as body mass, age, and sex may also be of relevance. Similarly, we can easily reduce risk by avoiding these drugs in patients with a high-to-moderate risk of cardiovascular disease. It would also be judicious to exclude from therapy with a selective COX-2 inhibitor patients with recognized prothrombotic environmental exposure (e.g., anovulants) or genetic (e.g., factor V Leiden) variants (116). However, given the biological plausibility of the time course of the results of the APPROVe and APC studies, we need to address seriously the possibility that prolonged treatment with coxib-like drugs may predispose gradually to an emerging hazard in those previously at low risk of cardiovascular disease. Does extended therapy with COX-2 inhibitors result in accumulation of atherosclerotic plaque burden in humans? If so, does some combination of biomarkers of drug exposure, mechanism-based risk transformation, and atherogenesis combine with physiological responses (such as the rise in blood pressure) and genetic variants to predict the small number of the individuals treated who progress to clinical events? Such information would then enhance the design of RCTs so that they might provide information of value to individual therapeutic decisions in the future. Simplistic approaches to trial design (117) are likely to be inconclusive. TARGET was announced as a trial in patients at high cardiovascular risk that would provide a definitive answer to the cardiovascular question (118). In fact, too few patients at high risk were recruited for the trial to be powered to exclude a cardiovascular risk from lumiracoxib (82). However, given present evidence, performance of an RCT involving a selective COX-2 inhibitor in high-risk cardiovascular patients (117) is, at the least, ethically questionable.

A regulatory approach that synthesizes available information in a manner most pertinent to clinical decision making. Both the FDA and the European Medicines Agency (EMEA) (http://www.emea.eu.int/) concluded that rofecoxib, valdecoxib, and celecoxib conveyed a small but absolute hazard of myocardial infarction and stroke. Rofecoxib and valdecoxib have been withdrawn from the market in the US, Europe, and other jurisdictions. Both agencies agreed that more information was desirable concerning tNSAIDs but reacted in a distinct, but important way. The FDA applied a "black box" warning to celecoxib, which remained on the market, but also to the tNSAIDs (119). The EMEA, in contrast, imposed restrictions on celecoxib (and on etoricoxib, which is on the market in some European countries) but concluded that there was no evidence to prompt a change in their advice about tNSAIDs (120). Adding a "black box" to the label of tNSAIDs is likely to mitigate the competitive damage to celecoxib and to diminish the hazard of litigation for all the relevant manufacturers by signaling risk to the consumer. However, an indiscriminate approach to warning about all remaining NSAIDs does not reflect the varied quality of the available evidence and is as practically valuable to patients and their doctors as the prior absence of explicit warning about the cardiovascular safety of any of these drugs.

Conclusions

Just as low-dose aspirin is effective in the secondary prevention of myocardial infarction and stroke and causes a small but definite risk of serious GI adverse effects (121), so selective inhibitors of COX-2 relieve pain and inflammation and convey a small but definite risk of myocardial infarction and stroke. While the preferential inhibition of COX-1 is *sufficient* to explain both the cardiovascular efficacy and adverse GI events observed with low-dose aspirin, so inhibition of COX-2 is *sufficient* to explain the antinflammatory efficacy and cardiovascular adverse events observed with the coxibs. Despite this, a plethora of additional actions of aspirin have been claimed to explain its action over the past 2 decades (122). The majority of these observations have been made in vitro and/or are of uncertain relevance to aspirin action at therapeutically tolerated doses in vivo. Similarly, a variety of alternative explanations for the cardiovascular effects of COX-2 inhibitors, again based largely on conjecture, in vitro data, and/or drug concentrations unlikely ever to be attained therapeutically have begun to emerge. Given the experience of aspirin, it might be wise to use the razor of William of Occam, the most celebrated proponent of the medieval principle of parsimony, to "shave off" unnecessary concepts, variables, and constructs. One should always choose the simplest explanation of a phenomenon, one that requires the fewest leaps of logic (123).

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