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Theme Issue Article

The pharmacology of selective inhibition of COX-2

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Summary

Selective inhibitors of cyclooxygenase (COX)-2 were developed to improve the safety of anti-inflammatory therapy in patients at elevated risk for gastrointestinal complications which are thought to be caused primarily by depression of COX-1 derived mucosal prostanoids. They were not expected to be more efficacious analgesics than compounds acting on both cyclooxygenases, the traditional (t) non-steroidal antiinflammatory drugs (NSAIDs). While these predictions were generally supported by clinical evidence, an elevated rate of severe cardiovascular complications was observed in randomized controlled trials of three chemically distinct COX-2 selective compounds. The cardiovascular hazard is plausibly explained by the depression of COX-2 dependent prostanoids formed in vasculature and kidney; vascular prostacyclin (PGI_2) constrains the effect of prothrombotic and atherogenic stimuli, and renal medullary prostacyclin and prostaglandin (PG) E_2 formed by COX-2 contribute to arterial pressure homeostasis. A drug development strategy more closely linking research into the biology of the drug target with clinical drug development may have allowed earlier recognition of these mechanisms and the cardiovascular risk of COX-2 inhibition. Open questions are i) whether the gastrointestinal benefit of COX-2 selective compounds drugs can be conserved by identifying individuals at risk and excluding them from treatment; ii) whether the risk extends to tNSAIDs; iii) and whether alternative strategies to anti-inflammatory therapy with a more advantageous risk-benefit profile can be developed.

Keywords

Clinical studies, atherothrombosis, atherosclerosis, inflammation, inflammatory mediators

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A multi-enzyme signaling cascade

NSAIDs, which include both traditional (t)NSAIDs and NSAIDs selective for COX-2, relieve pain, inflammation and fever by inhibiting the formation of bioactive prostanoids. The prostanoids, including prostacyclin (PGI_2), thromboxane (TxA_2), prostaglandin (PG) E_2, PGD_2 and $PGF_{2\alpha}$ are a class of bioactive lipids derived from the twenty-carbon molecule arachidonic acid (AA) (1). They are formed by the enzymatic activity of two evolutionary conserved (2) prostaglandin H synthases (PGHS), PGHS-1 and PGHS-2 (3). Both enzymes convert free AA, released from membrane phospholipids at the sn-2 ester binding site by the enzymatic activity of phospholipase A_2, to PGH_2. Catalytic function of the membrane anchored PGHSs requires dimerization, but just one active monomer (uninhibited by NSAIDs) within the complex is sufficient for PGH_2 formation (4). The reaction involves both cyclooxygenase (COX) and peroxidase activities within the PGHS enzymes (3), hence the PGHSs are commonly termed COX-1 and COX-2. The COX activity incorporates two oxygen molecules into AA or alternate polyunsaturated fatty acid substrates, such as linoleic and eicosapentaenoic acid. Metabolism of AA forms a labile intermediate peroxide, PGG_2, which is reduced to the corresponding alcohol, PGH_2, by the enzyme's hydroperoxidase (HOX) activity. Inhibitors of the PGHS enzymes, the tNSAIDs, the PGHS-2 selective NSAIDs and aspirin, block only the COX activity and are hence referred to as COX inhibitors – the term used in this article. Both tNSAIDs and NSAIDs selective for COX-2 inhibit the enzymes reversibly. Only aspirin acetylates serine[529] in COX-1 (or serine[516] in COX-2) covalently and inhibits enzymatic activity irreversibly. This unique feature, which sustains inhibition of platelet TxA_2 throughout the dosing interval, and the limited capacity of platelets for de-novo protein synthesis are thought to render aspirin the only COX inhibitor with proven cardioprotective activity (5), as new platelets have to be formed to restore function.

The unstable endoperoxide product PGH_2 is subject to further metabolism by isomerases and synthases which are ex-

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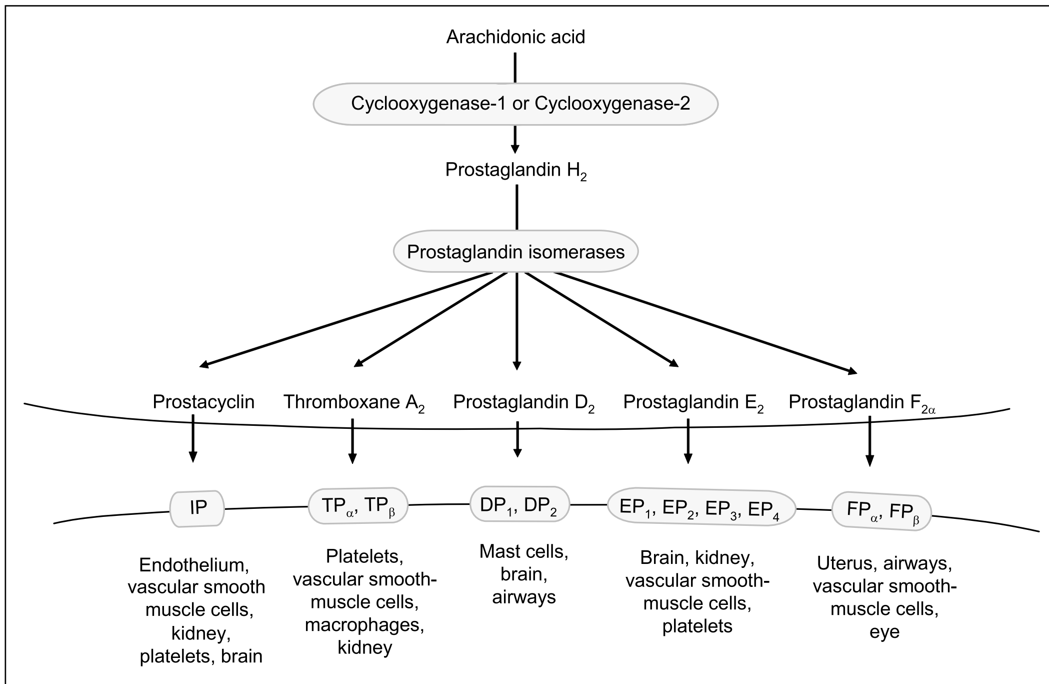

Figure 1: The cyclooxygenase (COX)-1 and –2 pathway. Prostaglandins (PG) are formed by specific isomerases from the COX product PGH_2. They act through G protein transmembrane receptors. IP, prostacyclin receptor; TP, thromboxane receptor; DP, PGD_2 receptor, EP, PGE_2 receptor, FP, $PGF_{2\alpha}$ receptor. (Reprinted with permission from Fries, Grosser. Hematology 2005; p. 445)

pressed in a relatively tissue-specific manner (1). These catalyze the formation of the active prostanoids. At least, nine such terminal synthases (Fig. 1) form the five biologically active AA cascade products (6, 7). Due to their short half-lives prostaglandins act as autacoids rather than circulating hormones by activating membrane receptors at, or close to, the site of their formation. Single receptors have been cloned for prostacyclin (the IP), $PGF_{2\alpha}$ (the FP) and TxA_2 (the TP), while four distinct PGE_2 receptors (the EPs 1–4) and two PGD_2 (DP1 and DP2) receptors have been identified (6, 7). These G-protein coupled receptors are characterized by different downstream signal transduction pathways. The EP2, EP4, IP and DP1 increase cAMP generation; the EP1, FP, and TP increase intracellular calcium levels. Splice variants of the EP3 can couple to both elevation of intracellular calcium and a decrease in cAMP (6, 8) and the DP2, a member of the fMLP receptor superfamily (9) decreases cAMP (10). Thus, activation of this intricate biosynthetic-response pathway by release of one common precursor, AA, can initiate a highly diverse array of signaling events, many of which are affected by differential inhibition of the COX enzymes.

The cascade is further complicated by the expression of both isozymes in some tissues including the vasculature and macrophages. An essentially unresolved question is how discrete signaling through the two isozymes is regulated in such cases, particularly when they are expressed within the same cell. Differences in their spatial distribution alone are not a sufficient explanation – despite of an enrichment of COX-2 in the nuclear envelope (11), as the subcellular expression patterns overlap considerably (12). A more likely distinction of the coexpressed isoforms relates to the biochemistry of the COX reaction, which requires activation of the heme group within the enzyme by peroxide-dependent oxidation. While the enzymatic kinetics of COX-1 and COX-2 are nearly identical, activation of COX-2 requires about 10-fold lower peroxide concentrations than COX-1 (13). Similarly, COX-1, but not COX-2, is subject to negative allosteric regulation, which may explain why higher concentrations of AA are required to initiate production of PGs by COX-1 than by COX-2 (14). These differences in the activation thresholds may render feasible independent functions of the two isoforms, even if they are expressed in the same cells. Recent evidence indicates that coexpressed COX-1 and COX-2 may also act in concert by forming heterodimers (15). Interestingly, selective inhibition of the COX-2 monomer within the protein complex may allow the physically associated COX-1 monomer to compensate for the inactivated COX-2 monomer (15), thus, retaining enzymatic function despite COX-2 blockade. These studies may explain tissue specific differences in the response to unselective versus COX-2 selective NSAIDs. They also accord with the observation that inhibition of the different COX isozymes within a cell or the same isozyme in different cells may have contrasting biological effects. Such studies into the relative roles of the two isoforms, however, lagged substantially behind the clinical development of the COX-2 selective compounds – not an uncommon observation in an industry where target discovery and clinical drug development are physically and intellectually separated. Indeed, when the race to developing selective inhibitors of COX-2 began – shortly after this isoform had been discovered (16–18), the majority of the molecular events downstream of the COXs and their biological functions had not been studied.

Inhibition of the COX-2 isozyme

A difference in the tertiary structures of the COX isozymes allowed the identification of compounds in screens of combinatorial libraries with higher affinity for COX-2 than COX-1 (Fig. 2). Subsequent crystallography revealed a hydrophobic

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pocket in the substrate binding channel of COX-2, which is absent in COX-1 (19, 20). Thus, selective inhibitors of COX-2 are molecules with side chains which fit within this hydrophobic pocket, but are too large to block COX-1 with equally high affinity. Several such compounds have been advanced into clinical development of which presently only celecoxib (Celebrex, Pfizer) and etoricoxib (Arcoxia, Merck) remain on European markets. Celecoxib remains the only 'coxib' on the US market. A newer substance, lumiracoxib (Prexige, Pfizer), is about to be marketed in the UK. Rofecoxib (Vioxx, Merck) and valdecoxib (Bextra, Pfizer) were withdrawn worldwide, when their cardiovascular risk was detected in randomized controlled trials (21–24). Currently, both the European Agency for the Evaluation of Medicinal Products (EMEA) and the United States Food and Drug Administration (U.S. FDA) have excluded from treatment with the remaining coxibs patients with cardiovascular disease and advise against the prescription to patients at elevated baseline risk.

The concept underlying the development of the coxibs – sometimes referred to as the 'COX-2 hypothesis' – assumed that the gastroduodenal toxicity of tNSAIDs was only related to their inhibition of COX-1-dependent PGE_2 and TxA_2 formation in gastric epithelium and platelets, while COX-2 had exclusive roles in pain mediation, inflammation and pyresis (25). Perhaps unsurprisingly, biological reality turned out to be more complex. Clearly, COX-2 is more readily inducible by inflammatory stimuli than COX-1 (17) and it is the major source of pain and inflammation mediating PGs (1). Unaccounted for by the 'COX-2 hypothesis', however, COX-1 can also be induced in inflammation – for example in the arthritic synovia (26) or in atherosclerotic plaque (27), and COX-2 is constitutively expressed in many uninflamed tissues (28–32). Similarly, both isoforms are developmentally regulated and coexpressed in some embryonic tissues (2). PGE_2 formed by COX-2 and the inducible microsomal PGE_2 synthase (mPGES)-1 is the predominant prostaglandin released at the site of tissue injury or inflammation and is thought to sensitize peripheral nociceptors. However, PGI_2 and perhaps TxA_2 play also roles in pain mediation (33). Both PGI_2 and PGE_2 contribute to another hallmark of inflammation, vasodilation. In addition to such peripheral mechanisms, COX-1 and COX-2 are both expressed centrally, in the spinal chord (34) where peripheral pain or inflammatory stimuli upregulate COX-2 in dorsal root neurons (35). Central nociceptive effects are thought to derive from the modulation N-methyl-D-aspartate (NMDA)-dependent neurotransmission (36).

Since predominantly COX-1 is expressed in normal gastric mucosa, selective inhibition of COX-2 was expected to impose a lower risk of gastric ulceration or bleeding than nonselective inhibition. Subsequently, low levels of COX-2 in healthy mucosa (37) and upregulation of COX-2 during acute stages of gastric erosion and ulceration were detected (38–40). Despite the possibility that such regulation of COX-2 might play a role in ulcer healing, the coxibs were approved based on their reduced rates of endoscopically visualized gastroduodenal ulcerations in comparison to equiefficacious doses of a tNSAID (41–43). Only three year-long (21, 44, 45) and one short-term (46) outcome studies have studied whether the coxibs actually reduce the incidence of serious gastrointestinal complications in larger populations.

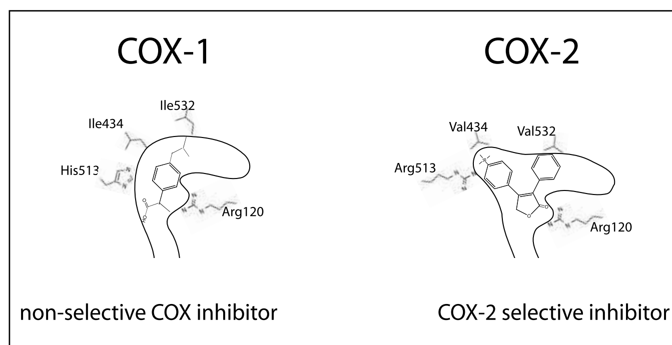

Figure 2: Schematic depiction of the structural differences between the substrate binding channels of PGHS-1 and PGHS-2 that allowed the design of selective inhibitors. The amino acid residues Val 434, Arg 513 and Val 523 form a side pocket in PGHS-2, while the more voluminous residues Ile 434, His 513 and Ile 532 in PGHS-1 obstruct access of the bulky side chains of the coxibs. Modified with permission from reference 54.

The Vioxx Gastrointestinal Outcomes Research (VIGOR) Study and the Therapeutic Arthritis Research and Gastrointestinal Event Trial (TARGET) have indeed shown that rofecoxib (Vioxx, Merck) and lumiracoxib (Prexige, Novartis), cause less serious gastrointestinal adverse events than non-isoform selective tNSAIDs (21, 45). By contrast, the long-term trial studying the oldest marketed COX-2 inhibitor, the Celecoxib Long-term Arthritis Safety Study (CLASS) study (47) failed to confirm the hypothesis, as its gastrointestinal endpoint did not differentiate this coxib from the comparator tNSAIDs. Interim data of CLASS at sixth months of treatment (44) and a more recent three-month trial (46), however, supported a favorable GI toxicity profile of celecoxib. Interestingly, celecoxib became the best selling coxib and remains the only such drug on the market in the US, despite of the weaker evidence for this compound's advantageous GI profile. Indeed, it seems worth remembering that the coxibs had been designed for a niche indication, to improve treatment safety for patients at high risk for gastrointestinal complications requiring chronic NSAIDs, a population of less then 5% of NSAID users (48). Prescription behavior changed, however, over time – driven by the appeal of an innovative therapeutic principle and an aggressive marketing strategy targeting both prescribers and consumers (49) – and more than a third of patients at the lowest risk for GI events received a COX-2 inhibitor in 2002 (48). Paradoxically, this occurred in a situation were the overall gastrointestinal complication rate was already declining probably due to prescription of lower doses of tNSAIDs and the increasing coadministration of gastroprotective agents (50).

The interaction of all NSAIDs (including both tNSAIDs and coxibs) with COX-1 and COX-2 is conditioned by their molecular structure and there is no absolute selectivity for one or the other isoform. Thus, selectivity, the relative affinities to COX-1 vs COX-2, is just as variable within the class as the chemical structures are. Indeed, COX-2 selectivity is best described on a continuous scale, on which all NSAIDs can be ranked. For example, the second generation compounds etoricoxib and lumiracoxib are more selective for COX-2 than rofecoxib and valdecoxib. Rofecoxib and valdecoxib are roughly similar in their de-

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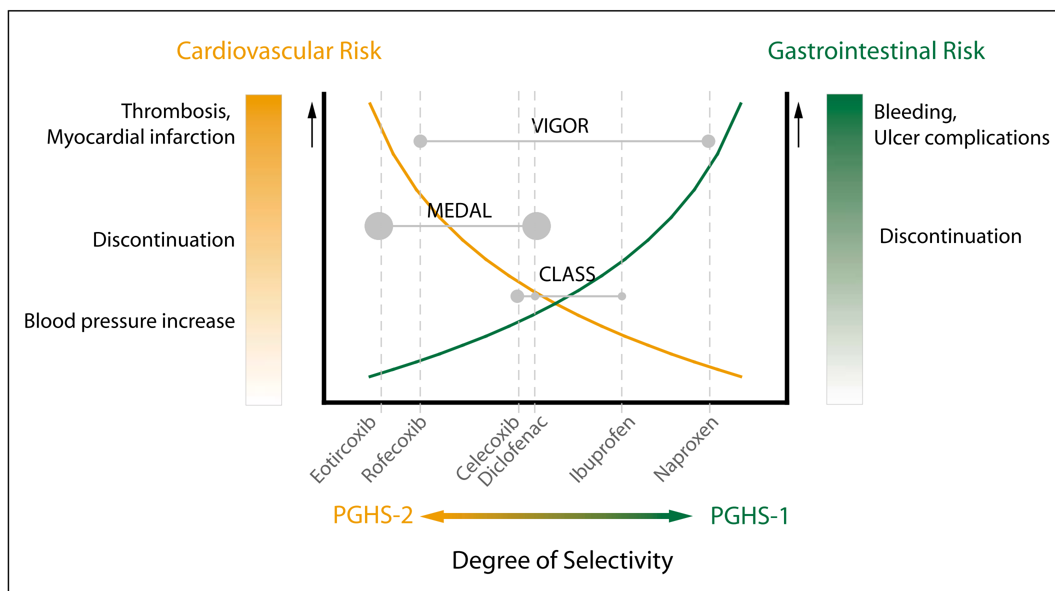

Figure 3: Implication of the relative degrees of selectivity. Increasing degrees of selectivity for COX-2 are associated with augmented cardiovascular risk, while increasing degrees of selectivity for COX-1 are associated with augmented gastrointestinal risk. Detection of the cardiovascular risk in randomized controlled trials is more likely the greater the difference is between the degrees of selectivity of the coxib and the tNSAID. A 'signal' has been detected in VIGOR but not in CLASS. The analysis of the MEDAL program is ongoing (115). Modified with permission from reference 54.

gree of selectivity, but more selective than celecoxib (51). It is not generally known that some of the older tNSAIDs, including diclofenac and meloxicam, are surprisingly similar in their degree of COX-2 selectivity to celecoxib. By contrast, substances like naproxen and ibuprofen are slighlty more potent inhibitors of COX-1 than of COX-2 (51). In human studies, the degree of COX-2 selectivity is commonly determined by whole blood assays which measure COX-1 and COX-2 inhibition ex vivo (52). We observed recently that 'selectivity' achieved in humans is not a purely structural property of a compound, but may also be influenced by pharmacokinetic (e.g. plasma concentration) and pharmacodynamic factors (e.g. genetic variations of the target enzymes) (53). Given that variable degrees of COX-2 selectivity are associated with variable degrees of gastrointestinal toxicity, one would expect a similar heterogeneity within the class of NSAIDs (tNSAIDs plus coxibs) of the cardiovascular safety profile. This is supported by mechanistic studies of COX biology and pharmacology [reviewed in (54)].

The cardiovascular biology of COX-2

The possibility of a cardiovascular hazard of COX-2 inhibitors was first hypothesized during late stage clinical development, when a marked depression of PGI_2 biosynthesis was observed in healthy volunteers (55, 56). A potent inhibitor of platelet function and vasorelaxing agent, PGI_2, was thought to act as a local restraint on prothrombotic stimuli (57). We obtained definite proof for this role of PGI_2 *in vivo* in genetic mouse models where disruption of PGI_2 signaling resulted in an augmented thrombotic response to endothelial injury (58). Mice deficient in PGI_2 signaling, however, were not prone to spontaneous thrombosis (33); the thrombotic process had to be induced by endothelial damage, but – once initiated – proceeded more vigorously than in mice with intact PGI_2 function. Projection of these observations into the clinical domain suggested that primarily patients with preexisting risk factors for thrombotic events, such as atherosclerotic vessel wall lesions or vascular inflammation, would be at risk for cardiovascular complications by COX-2 inhibition. It also suggested that the entire class of COX-2 selective compounds would augment cardiovascular risk through this unifying mechanism (59).

Two particular aspects of the model were disputed (60): i) The reduction of PGI_2 biosynthesis by the coxibs in man is not complete (55, 56, 61), unlike in mouse models, in which PGI_2 function was fully perturbed by homozygous deletion of the PGI_2 receptor, the IP (58). Indeed, COX-2 inhibitors depress PGI_2 by 50–70% in humans (55, 56, 61–63). Thus, the mouse model might not truly reflect human pharmacology. Subsequent experiments, however, showed that loss of a single copy of the IP – simulating a 50% decrease in PGI_2 formation – was sufficient to increase the susceptibility to thrombotic stimuli (64). Similarly, pharmacological inhibition of COX-2 in mice (64) and dogs (65) reduced PGI_2 biosynthesis and accelerated the arterial thrombotic response *in vivo*. Selective inhibition of COX-2 depressed PGI_2 biosynthesis also in rats and predisposed to platelet activation and arterial thrombosis under conditions of hypoxia-induced pulmonary hypertension (66). Likewise, selective inhibition of COX-2 enhanced platelet-vessel wall interactions in the hamster pouch (67) and, more recently, COX-2 deletion, which retains COX-1 as a source of PGI_2, enhanced platelet deposition in retinal vessels of hyperoxia treated mice (68). All these experiments demonstrate that partial inhibition of PGI_2, in the absence of coinciding platelet inhibition, augments the thrombotic process. ii) The second aspect relates to the source of PGI_2. PGI_2 biosynthesis is quantified by measurement of a stable urinary PGI_2 metabolite, 2,3 dinor 6-keto $PGF_{1\alpha}$ (PGI-M). This is an integrated measure of multiple sources of PGI_2 in the human organism and does not allow to map to the vessel wall the site of COX-2 dependent PGI_2 production with certainty (69). Thus, reduction of whole-body PGI_2 biosynthesis by selective inhibition of COX-2 may not necessarily reflect depression of synthesis in the vasculature. While the association of a biom-

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arker to a tissue source is never impeccable, studies *in vitro* showed that the endothelium is the major source of PGI_2 (70), and atherothrombotic vascular syndromes (57, 71) and iatrogenic vascular stimulation (72) all increase PGI_2 formation in humans. Native human (73, 74) and mouse arteries (75–77) express COX-2 in smooth muscle and/or endothelial layer. Indeed, the original COX-2 cDNAs were cloned from human, unstimulated endothelial cells (78, 79), suggestive of constitutive expression. Failure to detect vascular COX-2 expression consistently (80), may reflect dynamic regulation of COX-2 by flow-dependent mechanisms (77, 81) or perhaps by coagulation cascade proteins in the flowing blood (82, 83) not captured by the experimental procedure. It may also relate to the distinct activation thresholds of COX-1 and COX-2 (see above), which indicate that very low concentrations of COX-2 protein in the vessel wall may suffice for production of substantial amounts of PGI_2 (13, 14). This possibility is also supported by the large discrepancy between capacity of the COXs to produce PGs and actual biosynthesis of PGs in tissues, which may exceed three orders of magnitude (69, 84, 85).

A growing body of evidence suggests that the hazardous cardiovascular effects of selective inhibition of COX-2 are not limited to thrombosis, but may also involve direct effects on the vessel wall. Examination of the functional relevance of COX-2 in atherogenesis, however, has been limited by the reproductive and developmental defects of COX-2 deficient mice, which develop renal failure in early adolescence (86), and has relied primarily on pharmacological interventions. These have yielded variable results. Structurally distinct selective COX-2 inhibitors retarded atherogenesis in LDLR -/- mice when administered for eight weeks (87), accelerated lipid accumulation in aortic roots of ApoE -/- mice when administered for three weeks (88) and failed to modify atherogenesis in LDLR -/- (89), ApoE -/- (90), and LDLR/apobec-1 double knockout mice (91) when administered for 20–22 weeks. Bone marrow transplantion of COX-2 deficient donor mice accelerated early lesion development (87). Given the diverse biology of COX-2 products in blood pressure regulation (see below), its role in inflammation (92) and the limited understanding of the pharmacology of distinct COX-2 inhibitors in mice, it is perhaps unsurprising that pharmacological approaches have yielded varying results. Indeed, these observations may best be explained by the contrasting effects of COX products in various tissues relevant to the disease and their relative importance in distinct phases of the disease. Such tissue specific effects accord well with observations made with another AA metabolizing enzyme, 15-lipoxygenase-1 (15-LO-1). Tissues specific overexpression of the human 15-LO-1 in the endothelium accelerated atherogenesis in hypercholesterolemic mice (93), while tissue specific overexpression in macrophages protected from atherogenesis (94).

Given these observations, interest has shifted to the role of the individual pathway components downstream of the COXs. Disruption of the PGI_2 signal transduction pathway, by deletion of the IP, accelerated the initiation and early development of atherosclerosis in mice (95, 96). Absence of the IP augmented interactions of platelets and leucocytes with the vasculature and increased the attendant oxidant stress (95, 96). This was associated with an elevated biosynthesis of TxA_2, an index of platelet activation *in vivo* (96). By contrast, suppression of COX-1-drived TxA_2 activity retards atherogenesis (87, 91, 95) suggesting that COX-1-drived TxA_2 and COX-2-drived PGI_2 may have opposing activities in atherogenesis (95, 97). Opposing activities of these mediators imply that the degree of selectivity for COX-2 may condition the effects on atherogenesis – risk should increase with increasing selectivity. The intuitive concept of tipping a 'balance' between just these two molecules, however, is inept, as many vascular stimuli including thrombin, ADP and epinephrine oppose the activity of PGI_2 – just like TxA_2. While TxA_2 signaling was the first node to be studied as a modifier within this dynamic network (58), the roles of the other prothrombotic pathways including thrombin (98) and ADP signaling have yet to be assessed in this context. Describing the relationship between TXA_2 and PGI_2 as strictly binary might imply that low-dose aspirin would retain its full cardioprotective effect when administered concomitantly with COX-2-selective NSAIDs. Given the importance of the other prothrombotic pathways, however, it is more likely that COX-2 inhibition will offsets some of the protective effect of low-dose aspirin despite of complete suppression of TxA_2 formation. Thus, PGI_2's role would be more accurately described as a general constraint on prothrombotic and proatherogenic stimuli.

Elevation of blood pressure by COX-inhibitors may also accelerate atherogenesis and increase cardiovascular risk, albeit through a more indirect mechanism. Experiments in mice show that COX-1 and COX-2 have opposing functions in the kidney – just like in atherogenesis (99). Thus, COX-1 products, likely TxA_2 and perhaps $PGF_{2\alpha}$, contribute to blood pressure homeostasis by the renin-angiontensin system and increase arterial pressure (64, 100). Conversely, the vasodilator COX-2 products, PGI_2 and PGE_2, increase renal medullary blood flow, which drives diuresis and reduces blood pressure (101). Thus, inhibition of COX-2 by both tNSAIDs and coxibs lowers acutely medullary blood flow, sodium excretion and urine volume (99) and increases blood pressure in mice (64). Similarly, genetic perturbation of COX-2 increases blood pressure in mice (64). Given these distinct roles of the isozymes, one would again expect that the degree of selectivity of COX-2 inhibition would affect blood pressure control. This hypothesis is supported by a metaanalysis of approximately 45,000 patients in 19 clinical trials which suggests that selective inhibition of COX-2 elevates blood pressure more than non-selective inhibition (102). Similarly, the rate of hypertension in the Etoricoxib and Diclofenac Sodium Gastrointestinal Events (EDGE) study was increased in the group receiving the highly selective COX-2 inhibitor etoricoxib, in comparison to the less selective tNSAID comparator diclofenac (103).

Conclusions

Much of the COX biology was not well understood when development of the selective inhibitors of COX-2 began. Indeed, important components of this complex biosynthetic-response pathway still remain to be studied. Open questions pertain to functional interactions and distinctions of the two isoforms, particularly when they are expressed in the same cells, and to redundancies or differences in the molecular pathways downstream of the COXs. For example, selective suppression of PGE_2

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formation by inhibition of mPGES-1 (104) is being explored as a novel anti-inflammatory drug target, as this strategy may avoid the cardiovascular complications of the coxibs (64).

The possibility of a thrombotic cardiovascular risk of selective inhibition of COX-2 was not systematically addressed in clinical trials and such approach was not requested by regulators, even when biological and pharmacological evidence began to emerge during late clinical development (55, 56). Preapproval trials were too small and too short to detect the manifestations of the toxicity – thrombosis, myocardial infarction and stroke – by chance (51). Detection of a statistically discernable 'toxicity signal' would have required an unrealistic increase of drug induced cardiovascular events over the basal rate, because such thrombotic events were prevalent in the treated population at rates far above 'classical' severe toxicities such as hepatic failure (105). Perhaps unsurprisingly, the traditional pharmacovigilance systems failed even when millions of patients were exposed after drug approval. The cardiovascular toxicity 'signal' was detected by chance in the postapproval VIGOR Trial of patients with rheumatoid arthritis treated either with rofecoxib or naproxen for 12 months (21, 106). Four more randomized controlled trials have since detected a cardiovascular risk of three chemically distinct coxibs (22–24, 107, 108). The combined clinical experience is compatible with a prothrombotic effect of the coxibs in situations of elevated thrombotic risk (108) and a gradual increase of risk over extended periods of exposure (22, 107, 109). Indeed, the distinct roles of the COX isoforms in platelet function, atherogenesis and blood pressure control, provide a single, plausible mechanism by which selective inhibition of COX-2 may increase the likelihood of cardiovascular events in predisposed patients and in those initially at low risk who are exposed for extended periods of time (54). Mouse biology, human pharmacology, and clinical evidence all suggest strongly that the cardiovascular hazard pertains to all coxibs. They also suggest that differences within the class relate to the degree of selectivity achieved by individual compounds. We still know very little about the cardiovascular safety profiles of tNSAIDs, as prospective, placebo controlled trials have not been performed. However, their variable pharmacodynamic and pharmacokinetic properties suggest that they should not be perceived as a homogenous class; it is more likely that their cardiovascular safety profiles (110) are just as diverse as their gastrointestinal safety profiles, and some substances may indeed overlap with the selective inhibitors of COX-2. This has relevance for the detection of risk in controlled trials comparing a coxib with a tNSAID beyond the implications for patient care. Thus, comparison of a coxib with a relatively COX-2 selective compound such as diclofenac may favor the null hypothesis (Fig. 3) and mask absolute increases in cardiovascular risk. As we move towards an individualization of therapy, however, such variability of the pharmacodynmics and pharmacokinetics of NSAIDs may allow us to identify paradigms within which these drugs can be administered safely for extended periods in individuals at low cardiovascular risk (61).

Currently, changes in the drug approval process are being discussed as the consequence of the coxib experience. Proposals include an intensification of postmarketing drug safety programs, including more sophisticated pharmacovigilance and pharmacoepidemiological strategies and staged approval strategies with initial limitation to populations like those that were exposed during preapproaval trials (111, 112). Such strategies, however, still rely strongly on the prespecification of potential problems based on biological and pharmacological and clinical evidence. However, the integration of these different types of evidence remains an essentially unsolved problem. Criteria have been developed to objectify the quality of evidence derived from clinical studies (e.g. levels of evidence) (113, 114); however, information accumulated from cell biology, mouse genetics and biomarker studies is not formally assessed in its quality and systematically included in the evaluation. Only non-traditional approaches to drug development and academic research will be able to overcome such artificial boundaries between preclinical and clinical, basic and applied sciences and avoid failures as seen with the coxibs in future.

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