

Theme Issue Article

COX-2 inhibitors and cardiovascular risk

Inferences based on biology and clinical studies

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Summary

Even though non-steroidal anti-inflammatory drugs (NSAIDs) have been widely used for a long time, the search continues for anti-inflammatory drugs with few side-effects. COX-2 inhibitors are currently most debated, because they have less gastrointestinal side effects but have been linked to increased cardiovascular morbidity and mortality, presumably related to thrombotic events. This has brought about the withdrawal of rofecoxib and other COX-2 inhibitors from the market. Although the results

of several large studies with prospective, randomized design and meta-analysis of different trials have led to the demise of many popular COX-2 inhibitors, yet the conclusion seems to be rather simplistic. This review presents evidence from basic biology and clinical studies with the expectation that a balanced position, particularly in relation to increase in cardiovascular events, may be elucidated.

Keywords

Atherosclerosis, thrombosis, inflammation, hypercoagulability, coronary syndrome

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The cyclooxygenases

Cyclooxygenases (COXs) or prostaglandin endoperoxide synthases are enzymes responsible for the conversion of arachidonic acid into prostaglandins and thromboxanes (1). These eicosanoids have diverse effects in human biology affecting an array of organ systems, including gastrointestinal, reproductive, nervous, cardiovascular and blood coagulation systems. Their effects are mediated by cell surface receptors or intracellular nuclear receptors (2–5).

The purification of COX-1 (6) followed by the discovery of its isozyme COX-2 (7, 8) established their regulation and differential expression in various tissues. Information on the expression of COX-1 enzyme in many tissues and its constitutive activation (9) has led to the belief that this enzyme functions primarily as a “housekeeping” enzyme in the cytoprotection of gastric mucosa, regulation of renal blood flow and platelet aggregation (10–13). In contrast, activity of COX-2 is normally undetectable in blood vessels and platelets and is rapidly upregulated upon exposure to cytokines, endotoxins, tumor promoters and mitogens (7, 8, 14–17). This has contributed to the idea that

maintaining COX-2 mediates the production of inflammatory response prostanoids. However, COX-2 is constitutively expressed in the kidney and brain, suggesting that COX-2 may be necessary for maintaining certain organ functions (18).

Both COX-1 and COX-2 are integral proteins located mainly in the endoplasmic reticulum (19). They both possess bifunctional enzymatic activities, namely COX, which converts arachidonic acid into prostaglandin G₂ and peroxidase, which converts prostaglandin G₂ into prostaglandin H₂.

In terms of their specificity in producing eicosanoids, it is still not entirely clear which COX enzyme produces active eicosanoid/s. It appears that both enzymes can produce any type of eicosanoids depending on the presence of the corresponding eicosanoid synthase. COX-1 knock-out mice show markedly reduced prostaglandin E₂ in peritoneal macrophages, diminished inflammatory response to arachidonic acid stimulation, but not to phorbol acetate, reduced arachidonic acid-induced platelet aggregation and less indomethacin-induced gastric ulceration compared to the wild type mice (20). These in-vivo data convincingly support the well known functions of COX-1 from the in-vitro studies. These data also suggest that COX-1 inhibitor drugs

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are in fact quite specific, as previously thought.

Surprisingly, COX-2-deficient mice show normal inflammatory response to arachidonic acid and phorbol acetate, even though they show marked reduction in LPS-induced prostaglandin E_2 in peritoneal macrophage (21). This suggests that COX-2 may not be as critical in inflammation as previously thought. This study (22) also shows that COX-2 is more important in maintaining renal homeostasis, since the COX-2-deficient mice demonstrate severely impaired renal function. Interestingly, none of the COX-2 inhibitors have so far been shown to significantly affect renal function.

Recently, other isoforms of COX enzymes have been reported (22). They are spliced variants of COX-1 mRNA, i.e. COX-3, PCOX-1a and PCOX-1b. COX-3, was demonstrated to mediate the therapeutic effect of acetaminophen, which only weakly inhibits COX-1 and COX-2. Interestingly, not only COX-3 is more sensitive than COX-1 and COX-2 to inhibition by acetaminophen, it is even more sensitive to diclofenac, indomethacin, ibuprofen and aspirin. More importantly, COX-3 is mainly found in abundance in the heart and the cerebral cortex, suggesting the possibility of COX-3 mediated analgesic effect.

Platelets, endothelial cells and the cyclooxygenases

Thromboxane A_2 formed in the platelet by the action of thromboxane synthase possesses potent vasoconstrictor activity, facilitates cholesterol uptake, induces proliferation of vascular smooth muscle cells and stimulates platelet aggregation (23–25). On the other hand, prostaglandin I_2 (also known as prostacyclin) causes vasodilation, inhibits platelet aggregation, reduces cholesterol uptake and inhibits vascular smooth muscle cells proliferation.

Thromboxane A_2 is a prostanoid that induces platelet aggregation by binding to G-protein coupled receptor on platelet plasma membrane. Together with Gp VI/Fc γ R, Fc γ RIIA and integrin $\alpha_2\beta_1$, and GpIb/IX, thromboxane A_2 activates the signaling events mediated by phospholipase C β and phosphatidylinositol-3-kinase (PI3-kinase) resulting in the hydrolysis of plasma membrane phosphatidylinositol 4,5-bisphosphate (PI $_{4,5}P_2$) into diacylglycerol and inositol 1,4,5-triphosphate (IP $_3$) which causes intracellular Ca $^{2+}$ release from the smooth endoplasmic reticulum (26). Thromboxane A_2 induces the conformational change of the integrin $\alpha_2\beta_3$ which mediates the ultimate steps of platelet activation. All these events result in shape change, aggregation of platelets, and platelet binding to fibrinogen and fibronectin.

It is now evident that during systemic inflammatory states when there is an increased level of circulating cytokines, such as interleukin-1 β and tumor necrosis factor- α , the level of COX-2 expression in megakaryocytes increases. This leads to the expression of COX-2 in circulating platelets (27, 28). In fact, the presence of COX-2 at the mRNA and protein levels in circulating platelets in normal human subjects was first reported by Weber et al. (29).

The endothelial cells produce prostacyclin, a potent platelet inhibitor, when exposed to prostaglandin H_2 by the action of prostacyclin synthase (30). Under static conditions, endothelium expresses COX-1, but under physiologic stress develops upregu-

lated expression of COX-2 (31, 32). Since endothelial cells have a very active prostacyclin synthase, both COX isozymes may be responsible for the production of prostacyclin. It is still not entirely clear as to which COX enzyme or both is/are responsible for prostacyclin production by endothelial cells. Both COX-1 and COX-2 inhibitors have been shown to reduce the urinary excretion of 2,3-dinor 6-keto PGF $_{1\alpha}$, a prostacyclin metabolite (33). Thus COX-1 inhibitors reduce both thromboxane A_2 and prostacyclin synthesis. In contrast, COX-2 inhibitors do not reduce thromboxane A_2 but do reduce prostacyclin synthesis. Therefore, it has been hypothesized that the use of COX-2 inhibitors will result in unopposed thromboxane A_2 formation and platelet aggregation as prostacyclin production from endothelial cells is reduced (34). In theory, therefore, coxibs (selective COX-2 inhibitors) may selectively alter prostacyclin release and predispose patients to a pro-thrombotic state.

However, as discussed earlier, platelets also may express COX-2 during inflammatory stress and, therefore, thromboxane A_2 synthesis may also be inhibited by COX-2 inhibitors.

Cardiovascular risks of COX-2 inhibitors

There is an important role of platelet-endothelial interaction in the genesis of coronary atherosclerosis (35). It has long been believed that an excess of thromboxane A_2 formation results in platelet aggregation in the narrowed coronary arteries, which results in limitation of blood flow to the myocardium. If flow limitation is persistent, it results in myocardial infarction (MI). Indeed, enhanced thromboxane A_2 formation and platelet activity have been shown in patients with MI (36). Although intuitively one would think of diminished prostacyclin synthesis related to endothelial dysfunction, a characteristic of atherosclerosis, experimental studies have shown increased, rather than diminished, prostacyclin synthase activity in atherosclerotic arteries (37). Nonetheless, aspirin is frequently used in patients with MI to reduce platelet deposition in narrowed coronary arteries, and hence prevention of both primary and secondary cardiac events (38). It is noteworthy that commonly used doses of aspirin prevent formation of both thromboxane A_2 and prostacyclin, although smaller doses may have a selective effect on platelet thromboxane A_2 .

Some of the recent trials of coxibs have yielded provocative information on cardiovascular events occurring in patients taking these drugs (Table 1). It is noteworthy that these trials were conducted to determine their efficacy in the treatment of arthritic pain and their gastrointestinal safety. The results of these trials have been the subject of heated commentaries in press and lead to several multimillion dollar trials.

The initial large clinical trials with COX-2 inhibitors are the VIGOR and the CLASS studies. In the VIGOR trial, 8,076 patients with rheumatoid arthritis were randomized to rofecoxib 50 mg a day or 500 mg bid of naproxen followed for a median of nine months. The use of aspirin was an exclusion criterion. The primary outcome of this study was an upper gastrointestinal event. Mortality rate and death from cardiovascular causes were similar in both groups. Incidence of MI was slightly, but statistically significantly, increased in patients taking rofecoxib as compared to naproxen (0.4% vs. 0.12%) (39). The investigators

Table 1: Major clinical trials on coxibs.

| Trial | Patients | Drugs | Events | Ref. |
|--|--|---|--|------|
| I. ARTHRITIS TRIALS | | | | |
| VIGOR TRIAL | 8,076 patients with rheumatoid arthritis | rofecoxib 50 mg/d vs. naproxen 500 mg bid aspirin not allowed | MI risk higher in rofecoxib group compared to naproxen group (0.4% vs. 0.1%, RR 0.2, 95% CI 0.1 to 0.7) overall mortality and rate of death from cardiovascular causes were the same | 39 |
| CLASS Trial | 8,059 patients 28%:rh arthritis;72% osteoarthritis | celecoxib 400 mg bid; ibuprofen 800 mg tid, diclofenac 75 mg bid | CV events incidence similar in all groups, 0.9% vs. 1% in celecoxib vs NSAID (all) 0.5% vs. 0.4% in celecoxib vs NSAID (ASA) | 42 |
| TARGET trial | 18,325 patients with osteoarthritis | lumiracoxib 400 mg/d; naproxen 500 mg bid, ibuprofen 300 tid | Cardiac events in 0.65% of patients on lumiracoxib;0.55% on naproxen and ibuprofen groups combined | 45 |
| II. POST-CARDIAC SURGERY TRIAL | | | | |
| COX 2 inhibitors parecoxib and valdecoxib after cardiac surgery | 1,671 patients | IV parecoxib or oral valdecoxib, or IV placebo x3 d; then PO valdecoxib or placebo x10 days aspirin allowed (24% of patients took ASA) | Cardiovascular events more frequent on parecoxib – valdecoxib than those on placebo (2% vs 0.5%;RR 3.7, p=0.03) | 54 |
| III. COLORECTAL ADENOMA PREVENTION TRIALS | | | | |
| APPROVe Trial | 2,586 patients with colorectal adenomas to prevent progression | rofecoxib 25 mg daily (1287 patients) vs. placebo (1299 patients) aspirin allowed | 46 patients in rofecoxib group had thrombotic event vs. 26 patients on placebo; RR apparent 1.92 (CI-1.19–3.11) after 18 months of treatment | 53 |
| APC Trial | 2,035 patients, hx of colorectal ca | celecoxib 200 mg or 400 mg bid or placebo aspirin not allowed | 2.8–3.1 years follow up data (except for patients who died); CV event in 7 of 679 patients in placebo (1%); 16/685 on 200 mg bid (2.3%); 23/671 patients on 400 mg bid (3.4%) *CV event: MI, stroke, or heart failure | 52 |

Adapted with permission (63).

attributed the increased risk of this difference to the protective effect of naproxen against cardiovascular events which is a potent inhibitor of platelet aggregation (40). As the trial had no placebo group, this interpretation could not be verified. Importantly, rheumatoid arthritis is associated with an odds ratio of MI around 50% higher than patients with osteoarthritis or no arthritis (41), and inflammation is thought to be associated with a higher incidence of MI.

Celecoxib was studied in the CLASS trial (42). It was a randomized controlled trial of 8,059 patients with 28% of patients with rheumatoid arthritis, and 72% with osteoarthritis. Patients were randomly assigned to celecoxib 400 mg twice a day (n=3,987), or ibuprofen 800 mg three times a day (n=1985) or diclofenac 75 mg twice a day (n=1,996) for a six month study period. Aspirin use was allowed. The cardiovascular events were similar in celecoxib or ibuprofen or diclofenac groups regardless of aspirin use by patients. Incidence of MI in patients taking either celecoxib or NSAIDs varied between 0.3% to 0.5%. In patients not taking aspirin, incidence of MI in patients taking celecoxib or NSAIDs was about 0.1%. However, it should be noted that the original publication of the CLASS trial only contained data from the first six months of the trial and did not provide in-

formation regarding the use of diclofenac (43, 44). These discrepancies, as well as the study protocol which differed from that of the FDA, markedly limit the value of the CLASS trial.

A more recent large randomized controlled study was the TARGET trial. The study population was 18,325 patients with osteoarthritis, age 50 years or older. It compared lumiracoxib 400 mg once daily (n=9,156) against naproxen 500 mg twice daily (n=4754) or ibuprofen 800 mg three times a day (n=4,415). The primary cardiovascular endpoint was non-fatal and silent MI, stroke or cardiovascular death. The duration of the study was one year. There was no difference in cardiovascular end-points between lumiracoxib group (MI incidence 0.43%) and ibuprofen group (0.52%) in the ibuprofen sub-study. In the naproxen group, also there was no difference in MI rates in the lumiracoxib (0.84%) and naproxen groups (0.57%). There was also no significant difference whether the patients were taking aspirin or not (45).

In the CLASS and TARGET studies, no significant increase in cardiovascular events with COX-2 inhibitors compared to ibuprofen and diclofenac was found. But in the VIGOR trial, there was a significant increase of cardiovascular events compared to naproxen. In all these large trials, there was no placebo group;

therefore, it is hard to conclude whether diclofenac and naproxen have a protective effect against thrombotic events. It has been pointed out that none of the studies represent a real-world setting. The TARGET study excluded most patients with a prior history of cardiovascular diseases. Only less than 2% of the patients had previous MI or prior revascularization procedure. The TARGET trial was underpowered to assess cardiovascular risks (46). The use of intention to treat, absence of design to determine non-inferiority among the treatment groups, and no predefined upper confidence interval for relative risks have been highlighted as limitations in interpreting the result of this trial (34).

Other smaller or observational studies seemed to suggest an increased incidence and risks of cardiovascular events with the use of COX-2 inhibitors. Comparison of data from rofecoxib and celecoxib trials with earlier historical studies, which had used placebo as controls, showed that rates were higher with the two coxibs (47). In a nested case control study of 2,302,029 person-years of follow-up from the medical records at the Kaiser Permanente, high dose rofecoxib was found to increase the risk of serious coronary heart disease compared to celecoxib, with a 3.15-fold increased risk of MI and sudden cardiac death. Further, naproxen was found to not have any protective effect against serious coronary events (48). A case control study reported that rofecoxib 25 mg was associated with an elevated relative risk of MI compared with either celecoxib or no NSAID (49). An earlier retrospective study of medical records of the Tennessee Medicaid program also indicated that celecoxib users were 1.7 times more likely than non-users to have coronary events; in new users this rate increased to 1.93. Low-dose rofecoxib was not associated with increased incidence (50).

A cumulative meta-analysis of 18 randomized controlled trials and 11 observational studies comparing rofecoxib with any classical NSAIDs, which also included cohort and case-control studies, concluded that the risk of MI was increased 2.24-fold (51). Naproxen's protective effect was shown to be small and could not be explained by the findings of the VIGOR trial.

Results of the long awaited randomized, placebo controlled studies of COX-2 inhibitors were recently reported. Two of these studies were designed to look at the potential chemoprevention effect of COX-2 inhibitor in colorectal adenoma patients, the APC and APPROVe trials (52, 53). In the APPROVe trial, 2,586 patients with a history of colorectal adenomas were randomized to either 25 mg rofecoxib daily (n=1,287) or placebo (n=1,299). All potential cardiovascular serious events were adjudicated by an external committee blinded to the study. Results showed that rofecoxib was associated with an increased risk of cardiovascular events with relative risk of 1.92 after 36 months of treatment. However, overall mortality from cardiovascular causes was similar. During the first 18 months there was no difference between the two groups, but in the second 18 months, the difference became more apparent (53). Result of this trial facilitated the withdrawal of rofecoxib from the market. The second trial, APC trial, randomized 2,035 patients with a history of colorectal neoplasia to either celecoxib 200 mg (n=685) or celecoxib 400 mg per day (n=671) or placebo (n=679). Follow-up was done for 2.8 to 3.1 years. A composite end-point of cardiovascular events of death from cardiovascular causes, MI, stroke, or heart failure was used in this trial. Increased cardiovascular risk in a dose-dependent

fashion was reported, with an incidence of 1% in placebo and 2.3% in celecoxib 200 mg per day group and 3.4% in celecoxib 400 mg daily group (52). The overall absolute risk was 1.4% and would be better appreciated as a small increase in risk over a continuous treatment period. Other COX-2 inhibitors, valdecoxib and its intravenous prodrug parecoxib were compared in post-CABG patients to manage the pain. The primary endpoints included cardiovascular events besides renal insufficiency, gastroduodenal ulceration, and wound-healing complication. Patients received 10 days of therapy and were followed for 30 days. The results showed a significant increase in the incidence of cardiovascular events in COX-2 treated patients (54). These data delineated the danger of using COX-2 inhibitor in the post-CABG setting. After CABG, COX-2 levels have been reported to be increased in platelets (55, 56). It is possible that it is a protective mechanism in stress situation when inflammatory cytokines are released in the body. Potential inhibition of COX-2-mediated prostacyclin synthesis may have unmasked the pro-platelet aggregatory effect of platelet thromboxane A₂.

Interpretation of clinical trials in light of the biology of COX-2 inhibitors

COX-2 inhibitors were introduced not because of their strong analgesic effect, but because of their gastrointestinal tolerance. Based on the data from clinical trials, there is an indication that if taken continuously for a long period of time, these agents have a small, but definite risk of cardiovascular events. In its meeting on February 25, 2005, the FDA advisory committee recommended continued sales of celecoxib, valdecoxib and rofecoxib, although with considerable safety warnings.

COX-2 inhibitors have been in the vortex of controversy with the ups and downs following their introduction. The claim of their high specificity in blocking COX-2 enzyme without affecting COX-1 was initially thought to be the strength of the drugs. But later this was proven to be the main weakness of the drugs as these agents might leave the pro-thrombotic effect of COX-1 unopposed. However, this argument may not necessarily provide a complete understanding of the observations from the unpublished data from the ADAPT trial, a randomized, double-blind, multicenter trial of celecoxib 200 mg b.i.d. or naproxen sodium 220 mg b.i.d. vs. placebo for the primary prevention of Alzheimer's dementia, which suggested increased risks of cardiovascular and cerebrovascular events with naproxen, but not with celecoxib (full statement can be found at: <http://www.jhucc.com/adapt/documents.htm>). However, this trial could not be continued because of the result from the APC trial reporting the increased cardiovascular risk of celecoxib. The risk of cardiovascular events of naproxen in ADAPT trial is not conclusive, but it did provide a different conclusion on cardiovascular safety of celecoxib than the APC trial.

APC, APPROVe and valdecoxib in CABG patients trials suggested the cardiovascular risks with the use of COX-2 inhibitors, rofecoxib, celecoxib and valdecoxib. However, whether this is a class effect or limited to each compound is still unclear. The cardiovascular risks of etoricoxib are currently being studied in two major clinical trials, EDGE and MEDAL. MEDAL is studying

23,500 arthritis patients to compare the cardiovascular profile of etoricoxib to diclofenac. EDGE is studying the gastrointestinal effects of etoricoxib versus diclofenac in 4,000 arthritis patients. The final report has not been published, but the FDA advisory committee has released safety reviews on the drug (http://www.thepinksheet.com/FDC/AdvisoryCommittee/Committees/Arthritis+Drugs/021605_cox2day1/COX2preview3.htm) on its website. The preliminary data suggest increased risks of cardiovascular events, hypertension and heart failure, even though this is not enough to discontinue the study.

The use of aspirin with COX-2 inhibitor does not seem to alter the risks of cardiovascular events based on the data from CLASS, APC and APPROVe studies. This may be explained by the fact that patients taking aspirin usually already have high risks of cardiovascular disease, and these patients at high risk are not necessarily protected by the use of low-dose aspirin. Whether or not aspirin may prevent or alleviate the risks posed by COX-2 inhibitors in patients with low risks of cardiovascular disease could not be answered by these studies. Low-dose aspirin may balance the inhibition of COX-2 by inhibiting COX-1, but it may also increase the gastrointestinal side effects. Separate studies specifically designed to address these issues may be needed.

What transpires from what happened to these drugs seems to be the result of incomplete and oversimplified understanding of the biology of COX-1 and COX-2 enzymes and the not yet clear specificity of COX-2 inhibitors. The idea that COX-2 inhibitors may not be specific in their mechanism came mainly from the result of the COX-2 knock-out mice study. However, this study was unfortunately brushed aside during the discussion on the adverse

effects of these drugs (21) despite having been published by Dr. Smithies, whose scientific integrity and contributions are widely known by his discovery of the knock-out technology. His study in fact warrants a closer look at the role of COX-2 in in-vivo animal models and also necessitates confirmation of whether the coxibs block only COX-2 enzyme and have no other effects.

It should also be recalled that in the large clinical trials, COX-2 inhibitors were studied for their analgesic and anti-inflammatory effect, and later on for their effect on preventing adenoma formation. Focus on their adverse cardiovascular profile evolves from the observations of increase in MI risk, although there was no increase in overall cardiovascular mortality. Once the increase in MI risk was identified, the direction of "science" focused on explanation of the clinical observations, whereas previously a host of scientific observations pointed to their cardio- and vaso-protective properties (57–59). Since the hypothesis that COX-2 inhibitors block vasoprotective prostacyclin with unopposed thromboxane A₂ availability seemed logical in explaining elevated incidence of MI, this idea gained strength without much clinical evidence. It was forgotten that clinical studies one and a half decades earlier (60, 61) had shown no benefit of prostacyclin or thromboxane A₂ synthase or receptor blockers (62) in preventing or treating myocardial ischemia in man. It is important to continue to investigate the specificity of COX-2 inhibitors in inhibiting COX-2 and whether it is truly the underlying mechanism(s) of increased cardiovascular risks. In the meantime, due to its efficacy in alleviating pain with fewer GI side effects, it seems reasonable to maintain these drugs in the market with a package insert warning against long-term use.

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