Precaution, cyclooxygenase inhibition, and cardiovascular risk

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Cardiovascular risk led to the withdrawal of Vioxx (rofecoxib) in 2004. Some related drugs also increase cardiovascular risk and cyclooxygenase (COX)-2 inhibitors that remain on the market, including unselective non-steroidal anti-inflammatory drugs (NSAIDs), have not been exonerated. This article reviews if new evidence should change clinical and regulatory practice. Substantial COX-2 inhibition increases the incidence of cardiovascular disease unless concomitant platelet thromboxane production is inhibited by >95%. This can be investigated using whole blood assays, an approach used recently to show that acetaminophen (paracetamol) is a COX-2-selective inhibitor. The epidemiology available suggests acetaminophen, though readily available over-the-counter, does increase cardiovascular risk. Current evidence is inadequate to recommend many potential alternatives to Vioxx as safe. We argue that the precautionary principle, ‘first do no harm’, should underpin the regulation and prescribing of NSAIDs. Labelling which identifies these risks for prescribers and consumers should be mandatory.

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely prescribed group of therapeutic drugs worldwide, and include many common analgesics and anti-inflammatory agents. They inhibit cyclooxygenase (COX) enzymes, which accounts for desirable and undesirable effects (Figure 1). The pharmacological profile depends upon the dose and selectivity for COX-1 or COX-2 of the particular agent. COX-1-selective inhibitors reduce platelet TXA2 synthesis and can be as used as anti-thrombotic agents; they can also be anti-inflammatory and analgesic, but often cause gastrointestinal toxicity.

Non-selective COX-inhibitors are anti-inflammatory, analgesic and also cause gastrointestinal toxicity. COX-2-selective NSAIDs such as rofecoxib, valdecoxib, parecoxib and celecoxib, developed to reduce the burden of gastrointestinal toxicity, raise concerns regarding cardiovascular safety, though the mechanism is not known. Possibilities include increased arterial blood pressure, increased atherogenesis, or increased thrombotic tendency [1–5].

To reduce platelet aggregation, platelet TXA2 synthesis must be inhibited by >95% [6]. Low-dose aspirin achieves

this with little effect on basal prostacyclin biosynthesis, [4] and is also anti-thrombotic [7]. Prostacyclin, synthesised mainly by COX-2 [8], has opposing effects to those of TXA2. Its biosynthesis is increased in atherosclerosis where it is protective [2,3,5]. Moncada and Vane postulated a balance between thromboxane and prostacyclin thirty years ago, though this concept has been disputed [9]. A protective role for prostacyclin is supported by studies in mice deficient in the prostacyclin receptor [10], consistent with a prothrombotic action of COX-2 inhibitors through reduced synthesis of prostacyclin. COX-2 inhibition may also elevate blood pressure, accelerate atherosclerosis and undermine plaque stability [1,4]. PGE2, synthesised by COX-1 or COX-2, is a vasodilator, but may be proatherogenic [9].

The regulatory response to early signals of the cardiovascular risk of Vioxx [11] was tardy and limited [12]. Drug regulators and prescribers take a relaxed view of the safety of many NSAIDs, but the implications for patient safety are considerable. There are plausible mechanisms for cardiovascular toxicity of NSAIDs consistent with the primary pharmacology of COX-2 inhibition. Improved biochemical assessments predict the COX-1/2 profile of NSAIDs, which appears to predict cardiovascular risk accurately. The precautionary principle ‘first do no harm’ applies to European pharmaceutical regulation [13]. We argue that this should apply to NSAIDs as a class in terms of pharmacovigilance and prescribing, unless data from appropriate clinical trials provide sufficient reassurance for specific products. We propose that drug regulation should mandate labelling of NSAIDs that identifies these risks to prescribers and consumers. This opinion piece reviews how much cardiovascular morbidity and mortality might have been attributable to Vioxx and whether such toxicity is idiosyncratic, or is shared with other NSAIDs including acetaminophen.

Quantifying the cardiovascular morbidity and mortality associated with Vioxx

Vioxx was considered to be safe by the US Food and Drug Administration (FDA) in 2004, but withdrawn from the market by Merck in September that year because of potential cardiovascular toxicity. In 2005, a Texan court awarded $250 million to the widow of Robert Ernst, who died while taking the drug (http://news.bbc.co.uk/1/hi/business/4168332.stm). Estimates of the relative risk
of myocardial infarction while taking Vioxx vary; randomised controlled trials (RCTs) were designed to investigate protection to the gastrointestinal tract and adenoma prevention in patients with low cardiovascular risk rather than cardiovascular outcomes. Meta-analyses of such trials suggest a near doubling of myocardial infarction risk with Vioxx, with similar effects with other COX-2-selective inhibitors[14,15]. However, presentation of the original trial data has been questioned for the VIOXX Gastrointestinal Outcomes Research (VIGOR) trial[16] and Adenomatous Polyp Prevention on Vioxx (APPROVe) trial[17–19]. An alternative approach using nested controls showed substantial potential toxicity with an odds ratio for serious coronary heart disease for Vioxx of 1.47 (0.99–2.17) <25 mg/day and 3.58 (1.27–10.11) for >25 mg/day [20].

Epidemiological studies have too many potentially confounding factors to be convincing on their own [21], but data on cardiovascular risk are broadly consistent with the potencies of usual therapeutic doses of NSAIDs in inhibiting COX-2 [22]. This puts a greater suspicion on rofecoxib, celecoxib and diclofenac than on ibuprofen and naproxen.

The discussion of COX-2 inhibitors and safety has been clouded by legal issues and regulatory politics [23]. Cardiovascular toxicity cannot be excluded by pharmacovigilance because cardiovascular disease is so common (leading cause of death worldwide). This is in contrast to events such as phocomelia with thalidomide, where the incidence is so low that a signal is detectable against background noise [24]. The issue could have been answered if an RCT powered to detect cardiovascular endpoints had been launched in 1999 [12]. In the absence of such a trial, a meta-analysis of all RCTs of five coxibs indicated about one cardiovascular death per 1000 treated in one year [14]. About 80 million people worldwide took Vioxx [25], so this meta-analysis indicates about 80,000 excess deaths. This may be an underestimate because the recruited RCT populations were younger (mean age, ~58 years [11] compared with 68 years in a treated population [20]). Most of the RCTs excluded previous cardiovascular disease, patients who account for most of the increased risk [11]. Such exclusions are not replicated during use in clinical practice. One Vioxx trial [11] was criticised for missing data [16] and the myocardial event RR rose to 5.00 (95% 1.68–20.13) when three missing myocardial infarctions were included [16].

Given that RCT populations tended to be younger and excluded cardiovascular disease, observations in more general populations deserve consideration. US actuarial life tables predict approximately 700,000 cardiovascular deaths in one year in 80 million persons aged 65–70 years. If rofecoxib increased myocardial infarction fivefold [16],
then an excess of 2–3 million Vioxx-related cardiovascular events from a background of 700,000 expected cardiovascular deaths cannot be excluded. This is remarkable when compared to the modest benefit of anti-hypertensive or lipid-lowering agents, where halving the risk with drugs is unattainable in large trials in subjects at moderately increased cardiovascular risk. Halving cardiovascular risk in a population of this size and age might affect approximately 350,000 lives, so avoiding drugs such as Vioxx may be an even higher priority than prescribing drugs to prevent cardiovascular disease.

We review the trial data below and then discuss the population-based evidence. The epidemiology does not refute the possibility that a fivefold increase in myocardial infarction reported for Vioxx [16] could also apply to higher doses of some other COX-2 inhibitors. The TG1412 TeGenero Northwick Park disaster in 2006 [26] where a novel immunomodulator caused organ failure (but no deaths) in six previously healthy volunteers quite rightly led to a review of regulation. In contrast, the response to Vioxx did not change regulatory requirements for other NSAIDs. Whether this lack of a regulatory response is appropriate depends on whether or not the cardiovascular toxicity of Vioxx is a class effect of COX-2 inhibitors. Some adverse events stem from the primary pharmacology of a drug, shared by similar drugs as a class effect, whereas other adverse events are product-specific. Vioxx exemplifies the challenge to distinguish class effect toxicity from product-specific toxicity. Cardiovascular toxicity could be associated with COX-2 inhibition, or unique to Vioxx (e.g. attributable to a chemical structure that might promote atherogenic lipid peroxidation [27]).

Predicting cardiovascular toxicity of NSAIDs from a class effect of COX-2 inhibition

The pharmacology of prostaglandins (PGs) raises the possibility that drugs that leave thromboxane synthesis inhibited by <95% while inhibiting prostacyclin synthesis will predispose to heart attack and stroke [28].

Biochemical determination of drug effects on prostanooid synthesis in vivo has been problematic. A robust approach is to use an in-vitro COX selectivity test on blood sampled after drug administration in vivo (see Box 1) [29,30]. COX-1 activity is assessed from platelet thromboxane biosynthesis by clotting in vitro [31]. Thromboxane B2 (TXB2), the inactive product of TXA2, is measured in the serum. Incubation of whole blood with lipopolysaccharide (LPS) induces COX-2 in monocytes with increased synthesis of PGE2, a measure of COX-2 activity [22,32]. Garcia Rodriguez and colleagues used these methods to determine inhibition of whole-blood COX-2 by usual licensed doses of ten NSAIDs in healthy volunteers [22]. Naproxen inhibited platelet thromboxane synthesis by >95% and did not increase the RR of myocardial infarction in a nested case control analysis. The remaining nine traditional NSAIDs and coxibs did not reach this threshold value for TX inhibition. The RR of myocardial infarction observed in their General Practice database correlated (R² = 0.75) to the inhibition of whole blood COX-2 ex vivo. NSAIDs that inhibited COX-2 by <90% had a RR of myocardial infarction of 1.18 (95% CI: 1.02–1.38) whereas those with >90% COX-2 inhibition had a RR of 1.60 (1.41–1.81). An algorithm for the investigation of potential cardiovascular toxicity of NSAIDs is proposed in Figure 2.

Older data are consistent with the notion that such an algorithm could predict the cardiovascular profile of NSAIDs, though interpretation is complex [33]. Low-dose aspirin reduces cardiovascular risk and selectively inhibits platelet TXA2 synthesis by >95% [34–37]. In contrast, coxibs dose-dependently decrease PG synthesis while reducing TXA2 production by <10%, a pattern observed with rofecoxib [37–40], celecoxib [8,41], valdecoxib or its prodrug parecoxib [42–44], and etoricoxib [41,42,45].

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**Box 1. Techniques to measure COX activity in man**

**Background issues**

Simple assays such as radio-immuno assaying (RIA) readily quantify high concentrations of prostanooids in aqueous buffer, but low concentrations may be below the assay detection limit, and plasma or urine contain interfering substances.

Prostanoids are produced at low rates under basal conditions, but mechanical and other stimulation causes massive and rapid increases. Consequently, attempts to measure circulating prostanoids under basal conditions are bedevilled by low basal plasma concentrations and by unpredictable sampling artefact during venepuncture.

Measuring urinary metabolites avoids sampling artefact, but does not reveal the cell/tissue source of the prostanoid in question. Sampling of tissue from humans is limited by ethical constraints on invasive procedures.

**Advantages of measuring COX activity in blood**

Whole blood contains sources of both of COX-1 (platelets) and of COX-2 (monocytes). Stimulation of whole blood in vitro gives rise to such high concentrations of TXB2, a COX-1 product, and of PGE2, a COX-2 product from endotoxin-stimulated monocytes, that they can be assayed accurately after dilution with aqueous buffer using RIA or other simple high-throughput methods. Sampling artefact is not an issue as differences in plasma concentration are miniscule compared with the stimulated concentrations. COX-inhibitor or placebo is administered to the volunteer in vivo and venous blood sampled at intervals. The method [see reference 22 for original references] is well adapted for multiple dose studies with repeated sampling through the dose-interval, and may usefully be complemented by assaying plasma drug concentrations in parallel.

**Method of measuring COX-1 activity in response to endogenous thrombin**

Freshly drawn venous blood is divided into triplicate 1 mL aliquots in plain glass tubes at 37 °C and incubated for 60 min. After clotting serum samples are stored at –30 °C until convenient to assay. Thawed samples are diluted in buffer to ensure that the amount of TXB2 in a suitable volume for analysis falls on the log-linear portion of the standard curve, and measured in triplicate by a convenient method such as RIA.

**Method of measuring COX-2 activity in response to endotoxin (lipopolysaccharide, LPS)**

Freshly drawn venous blood is divided into triplicate 1 mL aliquots in tubes with 10 IU of sodium heparin and incubated both in the absence and in the presence of LPS (0.1–50 μg/ml) for 24 hours at 37 °C. Contribution from COX-1 is suppressed by adding aspirin (10 μg/ml) in vitro at time 0. Plasma is separated by centrifugation and kept at –30 °C. PGE2 is assayed in triplicate using RIA in thawed and suitably diluted samples.
coxibs are associated with cardiovascular risk, as outlined below.

Naproxen [37,46–48], ibuprofen [8,38,47,49] and diclofenac [38,41,50,51] significantly decrease TX as well as PGI₂ production. Naproxen and ibuprofen exhibit less cardiovascular risk than the coxibs in clinical trials and epidemiology, whereas the data for diclofenac are less reassuring.

**Evidence of cardiovascular toxicity of NSAIDs from randomized Phase-III trials**

To determine how prostanoid inhibition affects patient outcome requires clinical trial data. There is little commercial incentive to conduct large cardiovascular trials in the development of a new NSAID unless it is a regulatory requirement. After cardiac surgery patients are at high risk of myocardial infarction and this risk is increased with just 10-days treatment with valdecoxib and parecoxib: RR compared with placebo 3.7 (95% CI: 1.0–13.5) [52]. Most other trials excluded patients with previous cardiovascular events and the number of cardiovascular events was consequently low. Celecoxib use is associated with a dose-related cardiovascular risk [14,53,54], and differences between celecoxib and rofecoxib may be as much a matter of dose as difference in pharmacology. Within the meta-analyses of trials, allocation to rofecoxib, celecoxib, valdecoxib, etoricoxib or lumiracoxib is associated with a significant increase in serious vascular events relative to placebo (42%) with no heterogeneity between drugs [14]. Many of the trials within this analysis included one or two traditional NSAIDs as comparators. High doses of ibuprofen and diclofenac (but not naproxen) were also associated with an excess risk, similar to coxibs [55].

**Population-based cardiovascular risk and COX selectivity**

Epidemiology offers one way to determine if the risk measured in trials reflects risk in clinical practice. The disadvantage of a lack of blinded randomisation to treatment is offset by access to larger populations with fewer restrictions on inclusion; this is important because coxib risk may be greater for those with cardiovascular disease [56]. One observational database involved more than 70,000 women studied over 12 years in the Nurses’ Health Study [57]. These women, aged 44-69 years at baseline, provided medication data biennially. Data were analysed according to NSAID usage and hazard ratios (HRs) refined with multivariate analyses to account for confounding factors. Frequent NSAID use was associated with increased cardiovascular events, but low-frequency aspirin use was associated with reduced risk (multivariate RR 0.80; 95% CI, 0.68–0.95). Other NSAIDs or acetaminophen at high doses or high frequency were associated with an increased risk of major cardiovascular events [57]. The study is persuasive given its large size, careful documentation of medication, and long duration.

Mortality, hospital admissions and prescriptions for the entire population are recorded centrally in Denmark, and 4.6 million Danish citizens aged ≥10 years were included in a recent study [58]. A subpopulation of one million persons with no hospital admission in the previous five years and no co-medication for a range of major conditions was identified. HRs were computed for a composite of death and myocardial infarction, though this was primarily a mortality study. No excess risk was conferred in the population as a whole by ibuprofen or naproxen, but coxib use was associated with increased risk. Atherothrombotic disease is rare in young people, so the signal-to-noise cardiovascular event HR may be more robust in middle-aged subjects. In the 30–50-year-old group, the HR (95% CI) for ibuprofen was 1.76 (1.54–2.01), for naproxen 1.83 (1.30–2.63), for diclofenac 2.80 (2.35–3.34), for celecoxib 5.51 (3.93–7.74) and for rofecoxib 7.69 (5.67–10.43) compared with those who had not taken NSAIDs. These HRs are broadly consistent with the potencies of usual doses of these drugs as inhibitors of COX-2 and the >95% inhibition of COX-1 by naproxen [22]. If they represent true drug effects rather than confounding variables, they are
remarkable, particularly because the longest duration of treatment was only 34 days. It is hard to ascribe an effect over such a short time to an increase in blood pressure or progression of atheroma. A prothrombotic effect of COX-2 inhibition offers a plausible explanation. The risks associated with NSAIDs were dose-dependent, consistent with a pharmacological effect. While acknowledging the limitations of non-randomised data [21], such results should be explained rather than ignored, particularly because they are supported by a plausible pharmacological mechanism [50] and by the controlled trials mentioned above.

A lower HR of myocardial infarction for COX-2-selective inhibitors of 1.60 (95% CI 1.41–1.81) was reported in a retrospective review of a less inclusive UK General Practice database [22] and an approximate doubling of mortality in heart failure has been associated with rofecoxib, celecoxib and diclofenac [59]. These studies showed similar effects; differences between studies in the size of the RR may reflect differences between the populations studied.

**Acetaminophen (paracetamol) is a COX-2 inhibitor: implications for safety**

Much of the pharmacology of acetaminophen can be explained by selective inhibition of COX-2 [60–62]. Its mechanism is complex, and is affected by peroxide concentrations in the cellular environment [63]. In 1989, it was suggested that inhibition of prostacyclin synthesis by acetaminophen might be disadvantageous in myocardial infarction [64]. Acetaminophen in normal volunteers inhibits COX-2 by >80% in whole-blood assay, but does not block COX-1 sufficiently to affect platelet function or cause gastrointestinal toxicity [65]. This has led to a call for a review of the cardiovascular risk of acetaminophen [61].

In the absence of large cardiovascular trials of acetaminophen, the epidemiological evidence is patchy. In the Nurses’ Health Study, acetaminophen was associated with increased cardiovascular risk: adjusted RR 1.41 (95% CI 1.26–1.93) [57]. Phenacetin, of which acetaminophen is the active metabolite, has been associated with a RR of cardiovascular death of 2.9 (95% CI 1.5–5.5); salicylates in the same study showed no such effect [66], arguing against confounding by indication as an explanation.

**Conclusion**

The number of deaths caused by Vioxx may have been substantial. The subsequent debate raised uncomfortable safety issues for other COX-2 inhibitors. Clinical trials identified the cardiovascular risk of Vioxx, but the absence of such data for many other NSAIDs (including acetaminophen) is not reassuring. Overall, the data are consistent with the conclusion that substantial inhibition of COX-2, unless accompanied by inhibition of platelet TX production by >95%, increases the incidence of cardiovascular disease. The measurement of TXs and PGs in whole blood in small numbers of healthy subjects is fundamental for evaluating these drugs. This should prevent a repeat of the Vioxx experience. Labelling of NSAIDs, including acetaminophen, should reflect potential class-related cardiovascular risk, and risk–benefit assessment should follow the precautionary principle of ‘first do no harm’. Prescribers should bear in mind that to prevent cardiovascular disease, not prescribing high-risk drugs may be an even higher priority than prescribing drugs that lower cardiovascular risk.

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