Cell
PRESS

Precaution, cyclooxygenase inhibition, and cardiovascular risk

James M. Ritter[1], Idris Harding[2] and John B. Warren[3]

[1] Department of Clinical Pharmacology, King's College London School of Medicine at Guy's, King's College & St Thomas' Hospitals, St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, UK
[2] Division of Cardiac and Vascular Sciences, St George's Hospital, Blackshaw Road, London SW17 OQT, UK
[3] Top Flat, 25 South Audley Street, London W1K 2PB, UK

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 TXA_2 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 TXA_2 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 TXA_2. 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]. PGE_2, 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
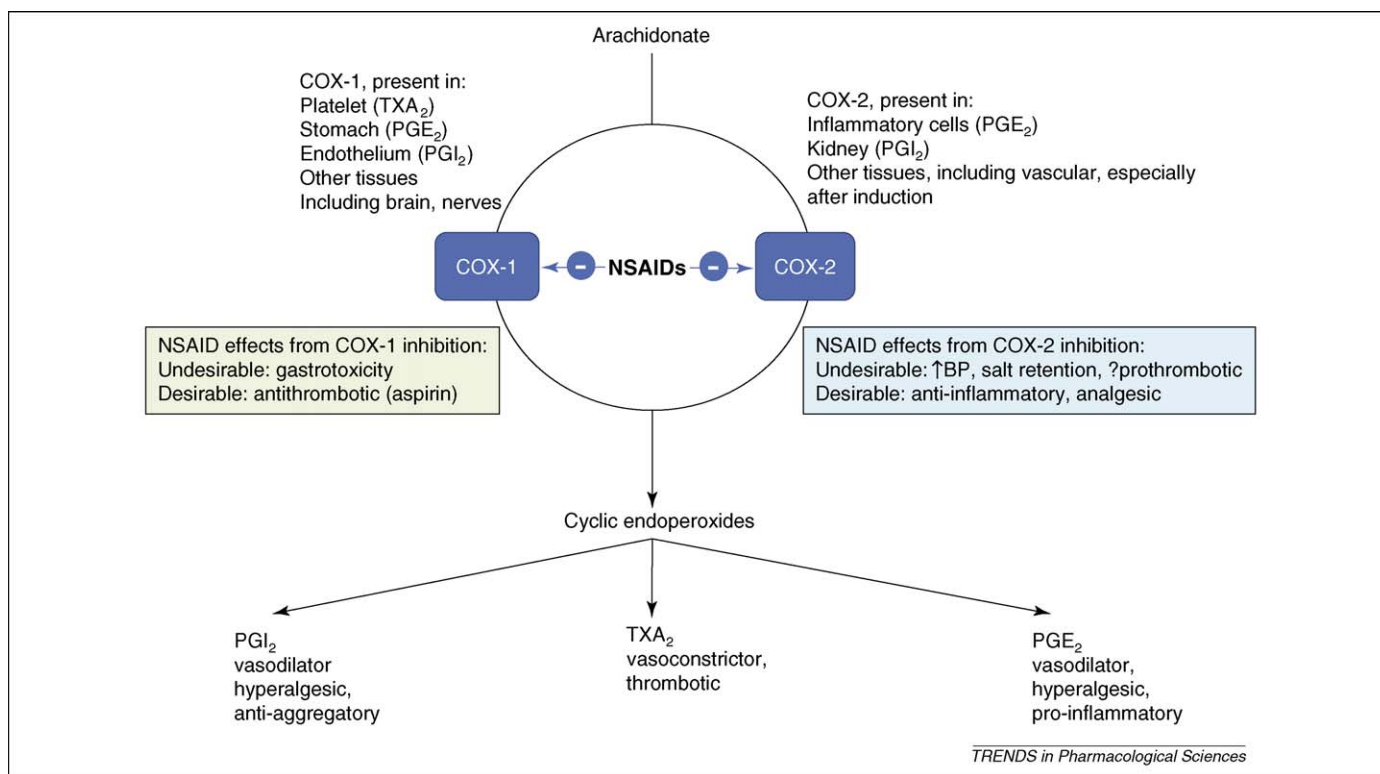
Figure 1. *Prostanoid synthesis and its inhibition by NSAIDs.* Arachidonic acid, present in esterified form in cell membranes throughout the body, is mobilised in response to a range of stimuli by phospholipase A_2 and oxidised to prostaglandin endoperoxide by cyclooxygenase (COX). COX converts arachidonic acid to endoperoxide PGG_2 and subsequently to endoperoxide PGH_2. PGH_2 is isomerised or converted enzymatically into prostanoids, the type of prostaglandin or thromboxane (TXA2) produced is determined by the cell, tissue and species distribution of specific synthase enzymes. In man, PGI_2 synthase is abundant in large blood vessels, especially in the endothelium, and in the renal glomeruli; PGE_2 is produced in the microcirculation, the stomach and renal medulla. TXA_2 is produced in platelets and released when they aggregate, making clotted blood a good model to study TxA_2 synthase inhibition. Two main isoforms of COX (COX-1 and COX-2) contribute to prostanoid synthesis. They are inhibited by NSAIDs, which differ in their COX isoform selectivity, accounting for much of the differences in pharmacology between NSAIDs. COX-1 is the only isoform present in platelets, and is also present in the endoplasmic reticulum of many tissues, including blood vessels. COX-2, found close to the nucleus, is induced by a range of mitogens and inflammatory stimuli, and may also be expressed constitutively, for example in renal macula densa. Endotoxin-stimulated blood is a good model to study COX-2 inhibition. Platelet synthesis of TXA_2 is reduced by COX-1 inhibition, prostaglandin production induced by inflammation is mainly reduced by COX-2 inhibition and gastroprotective prostanoids are reduced by COX-1 inhibition.

(RR) 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) $\leq$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],

---

Box 1. Techniques to measure COX activity in man

Background issues

Simple assays such as radio-immuno assay (RIA) readily quantify high concentrations of prostanoids 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 TXB_2, a COX-1 product, and of PGE_2, 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 TXB_2 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 (lipopolysacharide, 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. PGE_2 is assayed in triplicate using RIA in thawed and suitably diluted samples.

---

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 TGN1412 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 prostanoid 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 after stimulation by clotting *in vitro* [31]. Thromboxane B_2 (TXB_2), the inactive product of TXA_2, is measured in the serum. Incubation of whole blood with lipopolysaccharide (LPS) induces COX-2 in monocytes with increased synthesis of PGE_2, a measure of COX-2 activity [22,32]. Garcia Rodríguez 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 ($R^2 = 0.75$) 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 TXA_2 synthesis by >95% [34–37]. In contrast, coxibs dose-dependently decrease PG synthesis while reducing TXA_2 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]. These
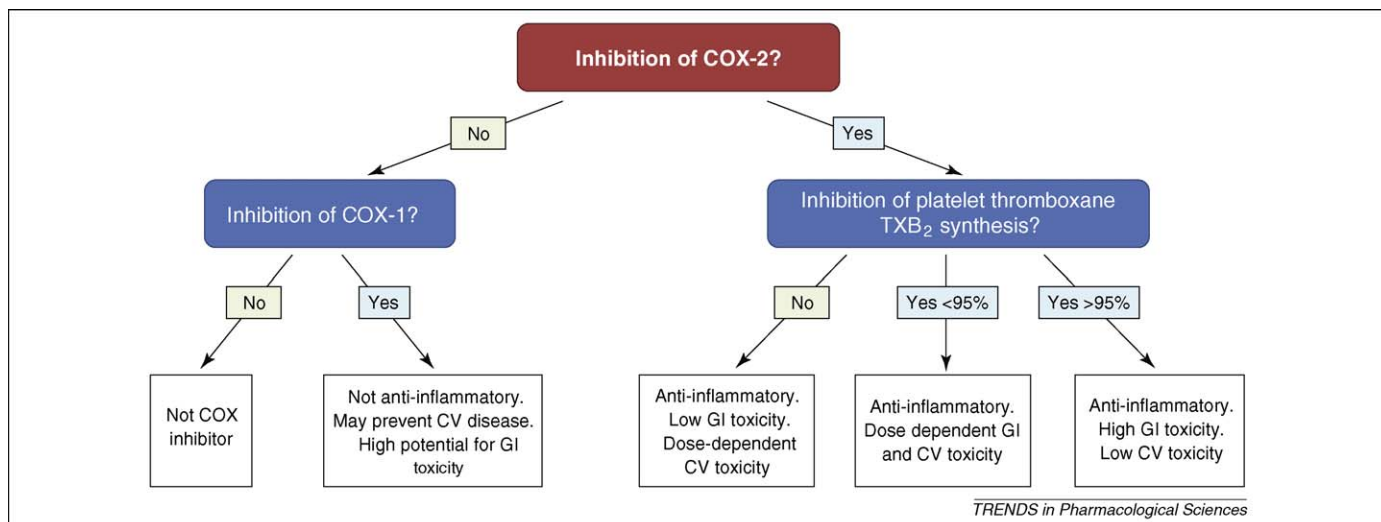
Figure 2. This algorithm gives broad guidance that should be interpreted with caution given that it does not take into account inter-individual variability. In addition, activity of NSAIDs is affected by their pharmacokinetic profile, which in turn is influenced by dosing, dosing frequency, and half-life of the drug and active metabolites: the methods of measuring COX-1 and COX-2 inhibition in whole blood outlined in the box lend themselves to a combined pharmacokinetic/pharmacodynamic approach with measurements of plasma drug concentration and effect on prostanoid synthesis throughout the dose interval.

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 PGI_2 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.

References

1 Egan, K.M. *et al.* (2005) Cyclooxygenases, thromboxane, and atherosclerosis: plaque destabilization by cyclooxygenase-2 inhibition combined with thromboxane receptor antagonism. *Circulation* 111, 334–342

2 Kobayashi, T. *et al.* (2004) Roles of thromboxane A(2) and prostacyclin in the development of atherosclerosis in apoE-deficient mice. *J. Clin. Invest.* 114, 784–794

3 FitzGerald, G.A. *et al.* (1984) Increased prostacyclin biosynthesis in patients with severe atherosclerosis and platelet activation. *N. Engl. J. Med.* 310, 1065–1068

4 Patrono, C. *et al.* (2005) Low-dose aspirin for the prevention of atherothrombosis. *N. Engl. J. Med.* 353, 2373–2383

5 Egan, K.M. *et al.* (2004) COX-2-derived prostacyclin confers atheroprotection on female mice. *Science* 306, 1954–1957

6 Reilly, I.A. and FitzGerald, G.A. (1987) Inhibition of thromboxane formation in vivo and ex vivo: implications for therapy with platelet-inhibitory drugs. *Blood* 69, 180–186

7 Antiplatelet Trialists' Collaboration (1994) Collaborative overview of randomised trials of antiplatelet treatment. I. Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. *BMJ* 308, 81–106

8 McAdam, B.F. *et al.* (1999) Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2. *Proc. Natl. Acad. Sci. U. S. A.* 96, 272–277

9 Flavahan, N.A. (2007) Balancing prostanoid activity in the human vascular system. *Trends Pharmacol. Sci.* 28, 106–110

10 Cheng, Y. *et al.* (2002) Role of prostacyclin in the cardiovascular response to thromboxane A$_2$. *Science* 296, 539–554

11 Bombardier, C. *et al.* (2000) Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. *N. Engl. J. Med.* 343, 1520–1528

12 Drazen, J.M. (2005) COX-2 inhibitors – a lesson in unexpected problems. *N. Engl. J. Med.* 352, 1131–1132

13 Asif, J. (2008) General Product Safety Law. In *The Law and Regulation of Medicines* (Feldschreiber, P., ed.), pp. 129–164, Oxford University Press

14 Kearney, P.M. *et al.* (2006) Do selective cyclo-oxygenase-2 inhibitors and traditional nonsteroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomized trials. *BMJ* 332, 1302–1308

15 McGettigan, P. and Henry, D. (2006) Cardiovascular risk and inhibition of cyclo-oxygenase: a systematic review of the observational studies of selective and nonselective inhibitors of cyclo-oxygenase 2. *JAMA* 296, 1633–1644

16 Curfman, G.D. *et al.* (2005) Expression of concern. *N. Engl. J. Med.* 353, 2813–2814

17 Bresalier, R.S. *et al.* (2005) The Adenomatous Polyp Prevention on Vioxx (APPROVe). Cardiovascular Events Associated with Rofecoxib in a Colorectal Adenoma Chemoprevention Trial. *N. Engl. J. Med.* 352, 1092–1102

18 Nissen, S.E. (2006) Adverse effects of rofecoxib. *N. Engl. J. Med.* 355, 203–204

19 Furberg, C. (2006) Adverse effects of rofecoxib. *N. Engl. J. Med.* 355, 204

20 Graham, D.J. *et al.* (2005) Risk of acute myocardial infarction and sudden cardiac death in patients treated with cyclo-oxygenase 2 selective and non-selective anti-inflammatory drugs: nested case control study. *Lancet* 365, 475–481

21 Moore, N. (2009) No obvious extra cardiovascular risk associated with low-dose NSAIDs. *Clin. Pharm. Therap.* (in press)

22 Garcia Rodriguez, L.A. *et al.* (2008) Role of dose potency in the prediction of risk of myocardial infarction associated with nonsteroidal anti-inflammatory drugs in the general population. *J. Am. Coll. Cardiol.* 52, 1628–1636

23 Patrono, C. and Rocca, B. (2009) Nonsteroidal antiinflammatory drugs: past, present and future. *Pharmacological Research* 59, 285–289

24 Warren, J.B. (2008) The Regulatory Process: pharmacovigilance in practice. In *The law and Regulation of Medicines* (Feldschreiber, P., ed.), pp. 129–164, Oxford, Oxford University Press

25 Topol, E.J. (2004) Failing the public health: rofecoxib, Merck, and the FDA. *N. Engl. J. Med.* 351, 1707–1709

26 Suntharalingam, G. *et al.* (2006) Cytokine Storm in a Phase 1 Trial of the Anti-CD28 Monoclonal Antibody TGN1412. *N. Engl. J. Med.* 355, 1018–1028

27 Reddy, L.R. and Corey, E.J. (2005) Facile air oxidation of the conjugate base of rofecoxib (Vioxx), a possible contributor to human toxicity. *Tetrahedron Lett.* 46, 927–929

28 Minuz, P. (2008) Nonsteroidal anti-inflammatory drugs and cardiovascular risk: is prostacyclin inhibition the key event? *J. Am. Coll. Cardiol.* 52, 1637–1639

29 Patrignani, P. *et al.* (1994) Biochemical and pharmacological characterization of the cyclo-oxygenase activity of human blood prostaglandin endoperoxide synthases. *J. Pharmacol. Exp. Ther.* 271, 1705–1712

30 Hinz, B. and Burne, K. (2008) Can drug removals involving cyclooxygenase-2 inhibitors be avoided? A plea for human pharmacology. *Trends Pharmacol. Sci.* 29, 391–397

31 Patrono, C. *et al.* (1980) Low dose aspirin and inhibition of thromboxane B2 production in healthy subjects. *Thromb. Res.* 17, 317–327

32 Brideau, C. *et al.* (1996) A human whole blood assay for clinical evaluation of biochemical efficacy of cyclo-oxygenase inhibitors. *Inflamm. Res.* 45, 68–74

33 FitzGerald, G.A. *et al.* (1983) Analysis of prostacyclin and thromboxane biosynthesis in cardiovascular disease. *Circulation* 67, 1174–1177

34 Patrignani, P. *et al.* (1982) Selective cumulative inhibition of platelet thromboxane production by low-dose aspirin in healthy subjects. *Clin. Invest.* 69, 1366–1372

35 Clarke, R.J. *et al.* (1991) Suppression of thromboxane A2 but not of systemic prostacyclin by controlled-release aspirin. *N. Engl. J. Med.* 325, 1137–1141

36 Rasmanis, G. *et al.* (1988) Effects of intermittent treatment with aspirin on thromboxane and prostacyclin formation in patients with acute myocardial infarction. *Lancet* 2, 245–247

37 Catella-Lawson, F. *et al.* (1999) Effects of specific inhibition of cyclooxygenase-2 on sodium balance, hemodynamics, and vasoactive eicosanoids. *J. Pharmacol. Exp. Ther.* 289, 735–741

38 Van Hecken, A. *et al.* (2000) Comparative inhibitory activity of rofecoxib, meloxicam, diclofenac, ibuprofen, and naproxen on COX-2 versus COX-1 in healthy volunteers. *J. Clin. Pharmacol.* 40, 1109–1120

39 FitzGerald, G.A. (2003) Cox-2 and beyond: approaches to prostaglandin inhibition in human disease. *Nat. Rev. Drug Discov.* 2, 879–890

40 McAdam, B.F. and Byrne, D. (2005) Contribution of cyclooxygenase-2 to elevated biosynthesis of thromboxane A2 and prostacyclin. *Circulation* 112, 1024–1029

41 Schwartz, J.I. *et al.* (2008) Comparative inhibitory activity of etoricoxib, celecoxib, and diclofenac on COX-2 versus COX-1 in healthy subjects. *J. Clin. Pharmacol.* 48, 745–754

42 Tacconelli, S. *et al.* (2002) The biochemical selectivity of novel COX-2 inhibitors in whole blood assays of COX-isozyme activity. *Curr. Med. Res. Opin.* 18, 503–511

43 Leese, P.T. *et al.* (2003) The COX-2 selective inhibitor, valdecoxib, does not impair platelet function in the elderly: results of a randomized controlled trial. *J. Clin. Pharmacol.* 43, 504–513

44 Graff, J. *et al.* (2007) Effects of parecoxib and dipyrone on platelet aggregation in patients undergoing meniscectomy: A double-blind, randomized, parallel-group study. *Clinical Therapeutics* 29, 438–447

45 Dallob, A. *et al.* (2003) Characterization of etoricoxib, a novel, selective COX-2 inhibitor. *J. Clin. Pharmacol.* 43, 573–585

46 Atherton, C.T. *et al.* (2004) Pharmacology and gastrointestinal safety of lumiracoxib, a novel cyclooxygenase2 selective inhibitor an integrated study. *Clin. Gastroenterol. Hepatol.* 4, 113–120

47 Scott, G. *et al.* (2003) Multiple dose lumiracoxib shows rapid absorption and COX-2 selectivity without accumulation in patients with rheumatoid arthritis. In Tulunay FC, Orme M, eds. European collaboration towards drug development and rational drug therapy. *Proceedings of the Sixth Congress of the European Association for Clinical Pharmacology and Therapeutics*. Berlin: Springer, 124, p197

48 Vesterqvist, O. and Gréen, K. (1989) Effects of naproxen on the in vivo synthesis of thromboxane and prostacyclin in man. *Eu. J. Clin. Pharmacol.* 37, 563–565

49 Hinz, B. *et al.* (2009) Lumiracoxib inhibits cyclo-oxygenase 2 completely at the 50 mg dose: is liver toxicity avoidable by adequate dosing? *Annals of the Rheumatic Diseases* 68, 289–291

50 Kirchheiner, J. *et al.* (2003) Pharmacokinetics of diclofenac and inhibition of cyclooxygenases 1 and 2: no relationship to the CYP2C9 genetic polymorphism in humans. *Br. J. Clin. Pharmacol.* 55, 51–61

51 Tegeder, I. *et al.* (1999) Comparison of inhibitory effects of meloxicam and diclofenac on human thromboxane biosynthesis after single doses and at steady state. *Clin. Pharmacol. Ther.* 65, 533–544

52 Nussmeier, N.A. *et al.* (2005) Complications of the COX-2 inhibitors parecoxib and valdecoxib after cardiac surgery. *N. Engl. J. Med.* 352, 1081–1091

53 Chen, Y.F. *et al.* (2008) Cyclooxygenase-2 selective non-steroidal anti-inflammatory drugs (etodolac, meloxicam, celecoxib, rofecoxib, etoricoxib, valdecoxib and lumiracoxib) for osteoarthritis and rheumatoid arthritis: a systematic review and economic evaluation. *Health Technol. Assess.* 11, 1–278

54 Solomon, S.D. *et al.* (2008) Cardiovascular risk of celecoxib in 6 randomized placebo-controlled trials: the cross trial safety analysis. *Circulation* 117, 2104–2113

55 Patrono, C. and Baigent, C. (2009) Low-Dose Aspirin, Coxibs, and other NSAIDS: A Clinical Mosaic Emerges. *Mol. Interv.* 9, 31–39

56 Baigent, C. and Patrono, C. (2008) Selective COX-2 inhibitors: where do we go from here? *Lancet* 372, 1712–1713

57 Chan, A.T. *et al.* (2006) Nonsteroidal antiinflammatory drugs, acetaminophen, and the risk of cardiovascular events. *Circulation* 113, 1578–1587

58 Fosbøl, E.L. *et al.* (2009) Risk of Myocardial Infarction and Death Associated With the Use of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) Among Healthy Individuals: A Nationwide Cohort Study. *Clin Pharmaco & Ther* 85, 190–197

59 Gislason, G.H. *et al.* (2009) Increased mortality and cardiovascular morbidity associated with use of nonsteroidal anti-inflammatory drugs in chronic heart failure. *Arch. Intern. Med.* 169, 141–149

60 Aronoff, D.M. *et al.* (2006) New insights into the mechanism of action of acetaminophen: Its clinical pharmacologic characteristics reflect its inhibition of the two prostaglandin H2 synthases. *Clin. Pharmacol. Ther.* 79, 9

61 Hinz, B. *et al.* (2008) Acetaminophen (paracetamol) is a selective cyclooxygenase-2 inhibitor in man. *FASEB J.* 22, 383–390

62 Li, S. *et al.* (2008) Acetaminophen: antipyretic or hypothermic in mice? In either case, PGHS-1b (COX-3) is irrelevant. *Prostaglan Other Lipid Mediat.* 85, 89–99

63 Boutard, O. *et al.* (2002) Determinants of of the cellular specificity of acetaminophen as an inhibitor of prostaglandin H2) synthases. *Proc. Nat. Acad. Sci. U.S.A* 99, 7130–7135

64 Green, K. *et al.* (1989) Pronounced reduction of in vivo prostacyclin synthesis in humans by acetaminophen (paracetamol). *Prostaglandins* 37, 311–315

65 Drvota, V. *et al.* (1991) Effects of non-steroidal anti-inflammatory drugs on the in vivo synthesis of thromboxane and prostacyclin in humans. *Adv. Prostaglandin Thromboxane Leukot. Res.* 21A, 153–156

66 Dubach, U.C. *et al.* (1991) An epidemiologic study of abuse of analgesic drugs: effects of phenacetin and salicylate on mortality and cardiovascular morbidity (1968 to 1987). *N. Engl. J. Med.* 324, 155–160