

# Risk management profile of etoricoxib: an example of personalized medicine

Paola Patrignani  
Stefania Tacconelli  
Marta L Capone

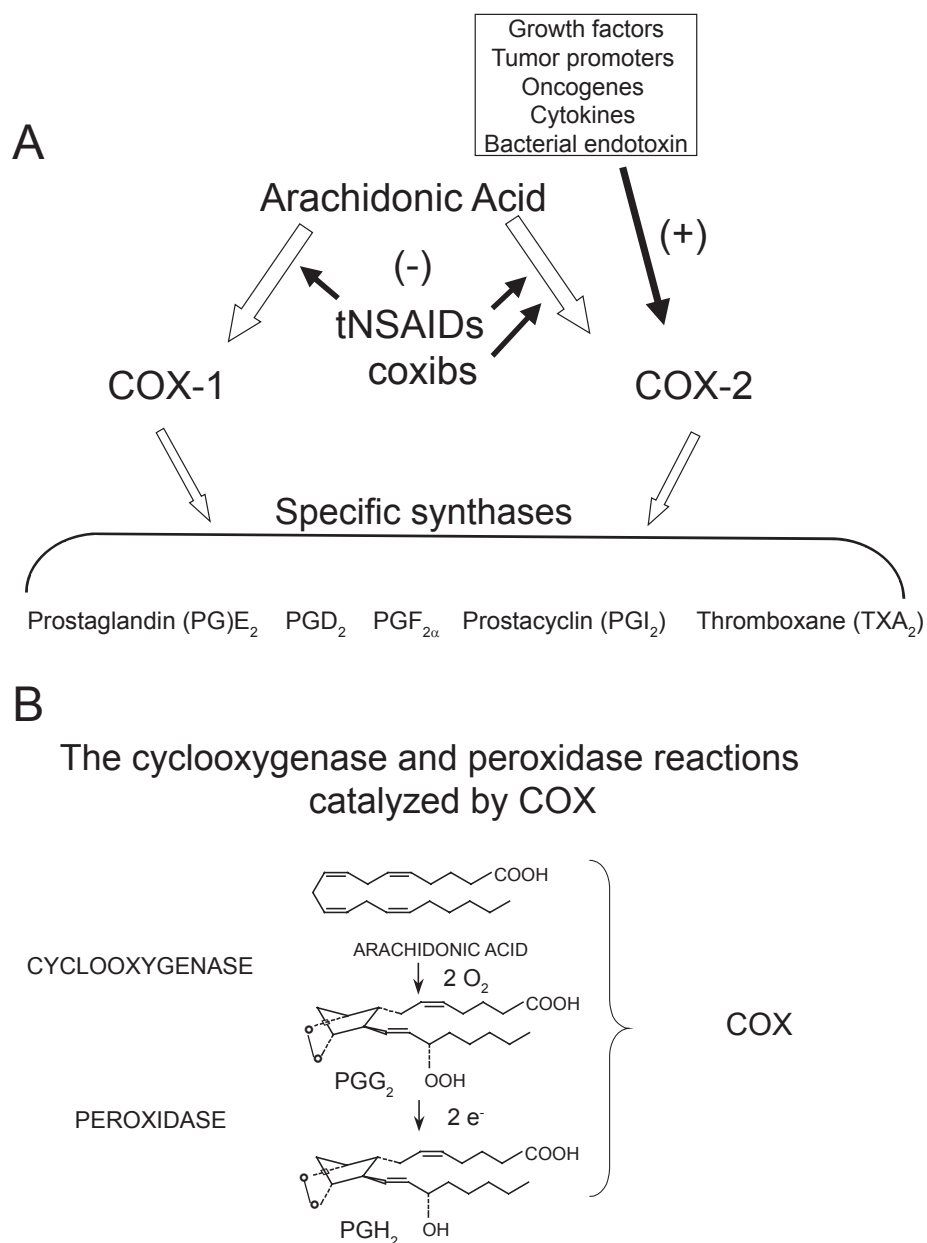
Department of Medicine  
and Center of Excellence on Aging,  
"G. D'Annunzio" University School  
of Medicine, and "Gabriele  
D'Annunzio" University Foundation,  
CeSI, Chieti, Italy

**Abstract:** The development of nonsteroidal anti-inflammatory drugs (NSAIDs) selective for cyclooxygenase (COX)-2 (named coxibs) has been driven by the aim of reducing the incidence of serious gastrointestinal (GI) adverse events associated with the administration of traditional (t) NSAIDs – mainly dependent on the inhibition of COX-1 in GI tract and platelets. However, their use has unravelled the important protective role of COX-2 for the cardiovascular (CV) system, mainly through the generation of prostacyclin. In a recent nested-case control study, we found that patients taking NSAIDs (both coxibs and tNSAIDs) had a 35% increase risk of myocardial infarction. The increased incidence of thrombotic events associated with profound inhibition of COX-2-dependent prostacyclin by coxibs and tNSAIDs can be mitigated, even if not obliterated, by a complete suppression of platelet COX-1 activity. However, most tNSAIDs and coxibs are functional COX-2 selective for the platelet (ie, they cause a profound suppression of COX-2 associated with insufficient inhibition of platelet COX-1 to translate into inhibition of platelet function), which explains their shared CV toxicity. The development of genetic and biochemical markers will help to identify the responders to NSAIDs or who are uniquely susceptible at developing thrombotic or GI events by COX inhibition. We will describe possible strategies to reduce the side effects of etoricoxib by using biochemical markers of COX inhibition, such as whole blood COX-2 and the assessment of prostacyclin biosynthesis in vivo.

**Keywords:** etoricoxib, nonsteroidal antiinflammatory drugs, COX-2, gastrointestinal toxicity, cardiovascular toxicity, prostacyclin

Nonsteroidal antiinflammatory drugs (NSAIDs) are commonly used in the general population for treating pain and inflammatory conditions (Burke et al 2006). They comprise traditional (t) NSAIDs and NSAIDs selective for cyclooxygenase (COX)-2 (named coxibs) which were developed to reduce the risk of serious gastrointestinal (GI) complications – dependent, at least in part, on the inhibition of COX-1 (FitzGerald and Patrono 2001). The therapeutic effects (analgesic and anti-inflammatory) of NSAIDs, both traditional and coxibs, are mostly due to the inhibition of COX-2-dependent prostanoids (Figure 1A). In placebo-controlled randomized clinical trials (RCTs), coxibs (rofecoxib [Vioxx®], celecoxib [Celebrex®, Artilog®, Solexa®, Artrid®] and valdecoxib [Bextra®]) were associated with an increase in the relative risk (RR) of cardiovascular (CV) events by 1- to 2.7-fold (Ott et al 2003; Bresalier et al 2005; Solomon et al 2005; Pfizer 2005; Nussmeier et al 2005). However, the results of observational studies and a meta-analysis of data derived from trials with coxibs have shown that the CV hazard is not restricted to NSAIDs selective for COX-2 but also applies to some tNSAIDs, such as diclofenac (Hernandez-Diaz et al 2006; Kearney et al 2006). In a recent nested-case control study, we found that patients taking NSAIDs (both coxibs

Correspondence: Paola Patrignani  
Dipartimento di Medicina e Scienze  
dell'Invecchiamento, Università di Chieti  
"G. D'Annunzio" and CeSI, Via dei Vestini  
31, 66100 Chieti, Italy  
Tel +39 0871 3556775  
Fax +39 0871 3556718  
Email ppatrignani@unich.it



**Figure 1** Pathways of prostanoid biosynthesis. (A) Prostanoids (PGE<sub>2</sub>, PGD<sub>2</sub>, PGF<sub>2α</sub>, PGI<sub>2</sub>, TXA<sub>2</sub>) are produced by COX-1 and COX-2 and specific synthases; (B) PGH<sub>2</sub>, generated by cyclooxygenase and peroxidase activity of COX, is then converted to prostanoids by the activity of different synthases.

and tNSAIDs) had a 35% increased risk of myocardial infarction (Patrignani et al 2008a). Clinical results suggest that the CV hazard associated with the administration of NSAIDs is dose-dependent (Patrignani et al 2008a; Solomon et al 2008). In addition, the genetic background of the individual may play a role in increased susceptibility to inhibition of NSAIDs (Arehart et al 2008). To limit the possible detrimental effects, associated with the administration of this efficacious class of drugs, is necessary to develop strategies of risk management through the identification of genetic and biochemical markers to select the

responders to NSAIDs or who are uniquely susceptible to developing thrombotic or GI events by COX inhibition.

### Differential COX pathways are effective in health and disease

#### Biology of COX-1 and COX-2

Prostanoids are lipid autacoids – including prostaglandin (PG) E<sub>2</sub>, PGF<sub>2α</sub>, PGD<sub>2</sub>, prostacyclin (PGI<sub>2</sub>), and thromboxane(TX) A<sub>2</sub> – that are immediately released outside the cell after intracellular biosynthesis and modulate a wide variety of

physiologic and pathologic processes via the interaction with specific receptors expressed mostly on the surface of target cells (Narumiya et al 1999; Breyer et al 2001).

Under normal physiologic conditions, prostanoids play an essential homeostatic role in the GI cytoprotection, hemostasis, renal physiology, gestation, and parturition (Funk 2001; Patrono et al 2001; FitzGerald 2003). Moreover, they play important roles in pathophysiological processes such as inflammation, cancer, and thrombosis (Funk 2001; Patrono et al 2001; FitzGerald 2003). Two isoforms of COX (COX-1 and COX-2) have been cloned and characterized (Simmons et al 2004). COX-1 and COX-2 are the products of different genes. COX-1 is considered a “housekeeping gene” by virtue of constitutive low-levels of expression in most cell types. However, high levels of constitutive expression of COX-1 have been detected in the stomach, platelets, and the kidney. In addition, COX-1 can be regulated during development (Rocca et al 1999). In contrast, the gene for COX-2 is a primary response gene with many regulatory sites; thus, COX-2 expression can be rapidly induced by bacterial endotoxin (LPS), cytokines, such as interleukin (IL)-1 $\beta$  and tumor necrosis factor- $\alpha$ , growth factors, and the tumor promoter phorbol myristate acetate (PMA) (reviewed by Kang et al 2007). However, COX-2 is constitutively expressed in some cells in lung (Asano et al 1996), brain (Yamagata et al 1993; Kaufmann et al 1997), kidney (Harris et al 1994), pancreatic  $\beta$ -cells (Robertson 1998), and GI carcinomas (Ristimaki et al 1997; Shao et al 2000; Carlson et al 2003).

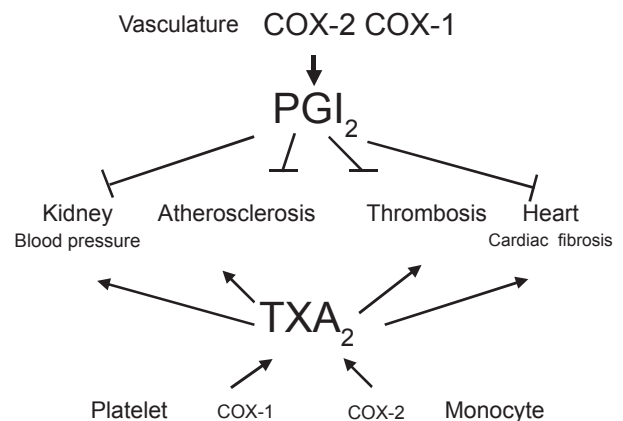
COX-1 and COX-2 share the same catalytic activities: the cyclooxygenase which oxidizes arachidonic acid (AA) to PGG<sub>2</sub> and the peroxidase which reduces PGG<sub>2</sub> to the unstable endoperoxide PGH<sub>2</sub>, precursor of prostanoids (Smith et al 2000) (Figure 1B). Although the peroxidase reaction is considered the second step in the formation of PGH<sub>2</sub>, the COX reaction is absolutely dependent on peroxidase activity for its activation (Malkowski et al 2000). However, the two COX-isozymes require different levels of hydroperoxides to initiate cyclooxygenase catalysis, ie, COX-2 requires considerably lower levels of hydroperoxides than COX-1 (Kulmacz and Wang 1995; Marnett et al 1999). Elegant work performed by Funk’s group reveals that COX-1 can partially compensate for COX-2 function but that this is limited by the differential ability of these two isoforms to metabolize low concentrations of arachidonate (Yu et al 2007). In fact, COX-2 catalysis occurs at lower levels of free AA than COX-1 (Swinney et al 1997).

These differences in the regulation of COX-1 and COX-2 catalysis contribute to the development of cell-specific pathways of prostanoid biosynthesis dominated by one or the

other enzyme *in vivo* even in the presence of the concurrent expression of the 2 isozymes (Capone et al 2007). As an example, in endothelial cells where different antioxidant defence systems are operative to maintain a reductive cytosolic environment, COX-2 is the dominant pathway turned on to generate prostacyclin, an important anti-atherogenic and anti-thrombotic mediator (Grosser et al 2006) (Figure 2).

## COX-2 is the major pathway in pain

Other than in inflammation and pyresis, COX-2 plays an important role in nociception (Vane et al 1998). Noxious stimuli (eg, physical, chemical, thermal) in peripheral tissues cause damage and the inflammatory response leads to the release of pain-producing substances that activate nociceptors on the terminals of sensory nerve fibers. This pain is the hallmark of protective response to adverse stimuli and subsides with removal of the stimulus. In this context, the induction of COX-2, leading to enhanced prostanoid release, translates into sensitization of peripheral nociceptor terminals and produces localized pain hypersensitivity. In fact, prostanoids increase neuronal activity in nociceptive nerve fibers by raising cAMP levels and lowering the activation threshold for opening of tetrodotoxin-resistant sodium channels in the neuronal membrane. PGE<sub>2</sub> and prostacyclin, produced in the peripheral terminals of sensory nerve endings, are hyperalgesic and enhance nociception produced by other mediators (such as bradykinin) (Murata et al 1997; Nakao et al 2007). Moreover, peripheral inflammation also generates pain hypersensitivity in neighboring uninjured tissue (secondary hyperalgesia), because of increased neuronal excitability in the spinal cord (central sensation) and a syndrome comprising diffuse muscle and joint pain, fever, lethargy, and anorexia (Samad et al 2001). NSAIDs play an



**Figure 2** Vascular prostacyclin counteracts the cardiovascular effects of platelet TXA<sub>2</sub>.

antinociceptive action by acting both at peripheral sites and at central sites mainly through COX-2 inhibition. Both COX-1 mRNA and COX-2 mRNA as well as COX-1 and COX-2 proteins are expressed constitutively in the spinal cord but only COX-2 can be induced in response to exogenous and endogenous cytokines (Beiche et al 1996; Inoue et al 1999; Tonai et al 1999). Like nociceptive pain, hyperalgesia is linked to an adverse stimulus and diminishes with healing and decreased inflammation. Prolonged acute pain and hyperalgesia, however, can evolve into chronic pain.

The results of RCTs showing a comparable pain relief by NSAIDs selective for COX-2 and tNSAIDs both in acute clinical pain models (for example, oral surgery) and chronic clinical pain models (such as osteoarthritis [OA] and inflammatory arthropaties), support a major role of inhibition of COX-2 in their clinical efficacy (Fitzgerald and Patrono 2001; Sciulli et al 2005). However, it should be pointed out that often the RCTs, comparing tNSAIDs and coxibs, had inadequate power to detect small differences possibly associated with inhibition of COX-1. Mostly, they were sized to detect equivalence, not superiority, of either (Patrono et al 2001).

## COX-1 is the major pathway in GI protection

Prostanoids (PGE<sub>2</sub> and prostacyclin) are key mediators to maintain gastric mucosal blood flow and increase protective mucus as well as bicarbonate production (Wilson 1991). Several lines of evidence show that prostanoids produced in normal GI tissue which are required for normal physiological functioning are derived from the COX-1 isoform (Kargman et al 1996). However, upregulation of COX-2 in the margin of healing ulcers and evidence from the use of COX inhibitors in rodents has raised concerns that COX-2 might be involved in the healing of existing ulcers (Shigeta et al 1998; Takahashi et al 1998). However, COX-2 up-regulation in the GI tract has been observed in cancer development (Romano et al 1998; Prescott and Fitzpatrick 2000; Nardone et al 2004; Wang and Dubois 2006).

## COX-1 is involved in TXA<sub>2</sub>-dependent platelet function

Platelets generate TXA<sub>2</sub> from AA through the activity of COX-1 (Patrignani et al 1999). TXA<sub>2</sub> generated in response to a variety of stimuli (for example, collagen, thrombin, and adenosine diphosphate) participates in the control of hemostasis through the induction of irreversible platelet aggregation triggered by the interaction with

G-protein-coupled receptors, the TXA<sub>2</sub> receptors (Alfranca et al 2006). Recent findings show that tiny concentrations of TXA<sub>2</sub> can cause platelet activation (Minuz et al 2002; Pulcinelli et al 2005; Minuz et al 2006). Thus, 10 nM of the TXA<sub>2</sub> mimetic U46619 induces platelet adhesion and shape change (Minuz et al 2002), and in the presence of a subthreshold concentration of collagen it causes platelet aggregation (Pulcinelli et al 2005). Moreover, TXA<sub>2</sub> causes vasoconstriction and vascular proliferation; thus, it is involved in atherogenesis and increase in blood pressure (Figure 2) (Grosser et al 2006).

## COX-2-dependent prostacyclin is the major pathway in vascular protection

In vascular endothelial cells, COX-2 plays a dominant role in producing important prostanoids to regulate the functions of underlying vascular smooth muscle cells and circulating cells. Although endothelial cells may generate a different array of the prostanoids PGD<sub>2</sub>, PGE<sub>2</sub>, and prostacyclin along the vascular beds, there is robust evidence that prostacyclin is the dominant prostanoid produced in the macrocirculation (Moncada et al 1977; Grosser et al 2006).

Prostacyclin potently inhibits aggregation of platelets induced by TXA<sub>2</sub> and other agonists, vascular smooth muscle cell proliferation and vascular tone, leukocyte-endothelial cell interactions, and cholesteryl ester hydrolase (Grosser et al 2006). As shown in Figure 2, most of the deleterious effects of TXA<sub>2</sub> in the CV system are countered by prostacyclin, mainly through the activity of COX-2. Thus, inhibition of vascular COX-2 leads to loss of the protective function associated with prostacyclin. Recently, it has been reported an antioxidant role for prostacyclin through the induction of hemoxygenase-1 (Egan et al 2004). For all these biological actions, prostacyclin has on the distinctive features of a cardioprotective mediator. Studies in knockout (KO) mice for prostacyclin receptor (IP) and the recent findings of acceleration of CV disease in humans by a dysfunctional IP mutation convincingly support the protective role of prostacyclin for the CV system (Arehart et al 2008; Patrignani et al 2008b).

## Biochemical biomarkers of COX inhibition

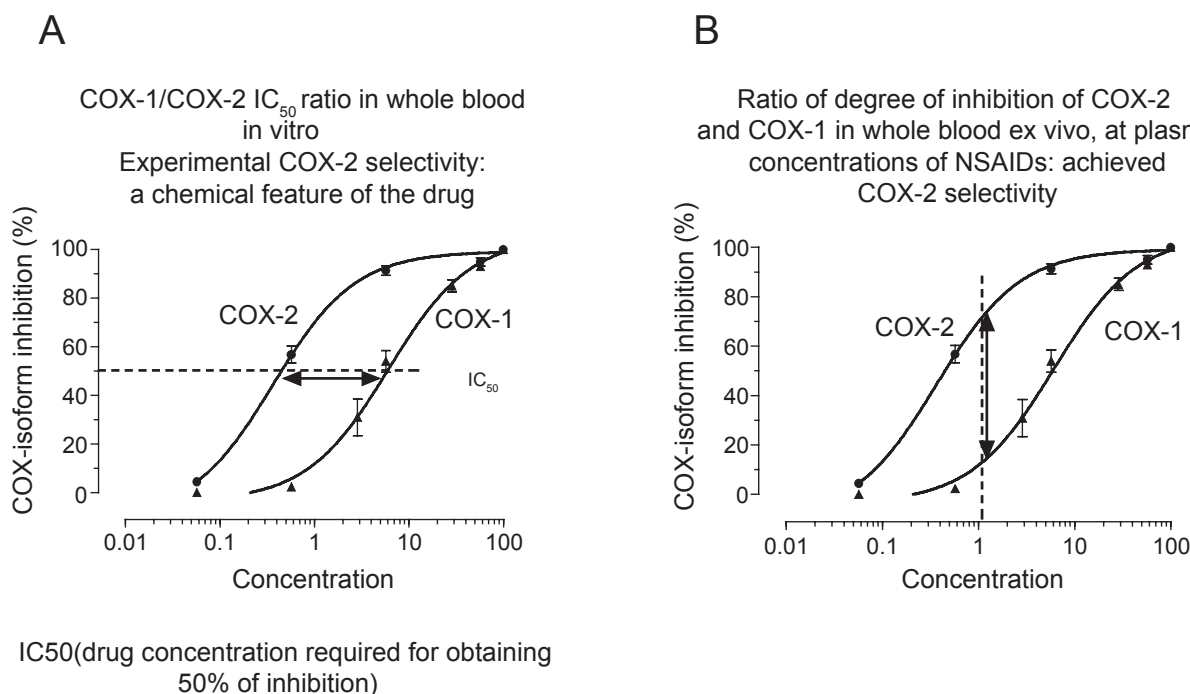
NSAIDs are distinguished on the basis of their COX-isozyme selectivity *in vitro*, described as the ratio of the concentrations required to inhibit the activity of the isozymes by 50% (IC<sub>50</sub> for COX-1/IC<sub>50</sub> for COX-2) (Figure 3A). The assessment of COX-1/COX-2 ratios *in vitro* describes an experimental

COX-isozyme selectivity which mirrors the chemical features of the different NSAIDs. This is assessed using the human whole blood assays which evaluate the effects of drugs on platelet COX-1 and monocyte COX-2 activities (Patrino et al 1980; Patrignani et al 1994). They are capacity indexes of COX-isozyme activities to generate prostanoids from endogenous sources of AA and their pharmacological inhibition is not influenced by different pathological conditions. These assays are based on the measurement of PGE<sub>2</sub> production, in response to lipopolysaccharide (LPS) added to heparinized human blood samples for 24 h which reflects the time-dependent induction of COX-2 in circulating monocytes (Patrignani et al 1994). The parallel measurement of TXB<sub>2</sub> production during whole blood clotting is used as an index of platelet COX-1 activity (Patrino et al 1980). When the COX-1/COX-2 IC<sub>50</sub> ratio is higher than 1 the drug is more potent in inhibiting COX-2. When the ratio is approximately 1, the drug is a non-selective inhibitor of both COX-1 and COX-2. When the ratio is lower than 1, the drug is more potent towards COX-1. We assessed COX-1/COX-2 IC<sub>50</sub> values of several COX inhibitors. Interestingly, we have found that the biochemical selectivity is a continuous variable preventing the separation of tNSAIDs from coxibs (Patrignani et al 2008c). However, we can identify some drugs more selective for COX-1 than COX-2 (such as aspirin, naproxen and ibuprofen) from the wide cluster of agents more

selective for COX-2 than COX-1. This includes: i) piroxicam and indomethacin which are approximately 3-fold more selective for COX-2; ii) etodolac, meloxicam, diclofenac, celecoxib, and valdecoxib which are 6- to 60-fold more selective for COX-2; iii) the highly selective COX-2 inhibitors such as, etoricoxib, rofecoxib, and lumiracoxib which have a COX-1/COX-2 IC<sub>50</sub> ratio higher than 100 (Table 1).

The whole blood assays also enable estimation of achieved COX-isozyme selectivity in humans, which is the ratio of isozyme inhibition at a given plasma concentration (Figure 3B). Importantly, achieved selectivity of NSAIDs varies as a consequence of the dose administered.

There is evidence that the whole blood assays for COX-2 and COX-1 may be candidate surrogate end-points of efficacy and GI toxicity of NSAIDs, respectively. It has been shown that COX-2 inhibition, as determined by PGE<sub>2</sub> levels in LPS-stimulated whole blood, can be used as a marker to predict drug efficacy in humans (Huntjens et al 2005). In fact, IC<sub>80</sub> (concentration required to inhibit the activity of COX-2 by 80% in vitro) values have been found to correlate directly with the analgesic/anti-inflammatory plasma concentrations of different COX inhibitors (Huntjens et al 2005). Thus, COX-2 inhibition, by assessing PGE<sub>2</sub> levels in the whole blood assay in vitro and ex vivo, can be used as a marker to predict drug effects (analgesia, antiinflammatory effects) in humans. Similarly, platelet COX-1 inhibition, by assessing



**Figure 3** Assessment of COX-isoform selectivity in whole blood in vitro (A) and ex vivo (B).



**Table 1** Biochemical selectivity, assessed as COX-1/COX-2 IC<sub>50</sub> values of several COX inhibitors (derived from Patrignani et al 2008a)

| COX-inhibitors | COX-1/COX-2 IC <sub>50</sub> values |
|----------------|-------------------------------------|
| naproxen       | 0.49                                |
| ibuprofen      | 0.56                                |
| indomethacin   | 2.9                                 |
| piroxicam      | 3.1                                 |
| etodolac       | 5.6                                 |
| meloxicam      | 13.8                                |
| diclofenac     | 24.4                                |
| celecoxib      | 32                                  |
| valdecoxib     | 61 <sup>a</sup>                     |
| etoricoxib     | 162                                 |
| rofecoxib      | 255                                 |
| lumiracoxib    | 400 <sup>b</sup>                    |

<sup>a</sup>COX-1/COX-2 IC<sub>50</sub> ratio value from Capone et al 2008.

<sup>b</sup>COX-1/COX-2 IC<sub>50</sub> ratio value from Patrignani et al 2004.

TXB<sub>2</sub> levels in the whole blood assay in vitro and ex vivo, can be used as a marker to predict toxicity or efficacy (GI toxicity or antiplatelet effects) in humans by NSAIDs. In fact, Cryer and Feldman (1998) reported that the inhibitory effects of tNSAIDs on gastric PGE<sub>2</sub> synthesis correlate with COX-1 inhibitory potency in clotting blood.

Now it is critical to establish the CV safety profile of specific individual NSAIDs, be it tNSAIDs or coxibs, and to find valid surrogate biochemical predictors of that risk.

Studies of clinical pharmacology have shown discordant functional implications of inhibition of COX-1 and COX-2. A linear relationship exists between the extent of inhibition of COX-2 and that of prostacyclin in vivo (Patrignani et al 2008a). In contrast, inhibition of TXA<sub>2</sub>-dependent platelet function in vivo occurs when platelet COX-1-dependent capacity to synthesize TXA<sub>2</sub> (as assessed by measuring serum TXB<sub>2</sub> levels) is reduced  $\geq 95\%$  (Reilly and FitzGerald 1987). In fact, a non-linear relationship of inhibition of platelet TXA<sub>2</sub> generation with inhibition of TXA<sub>2</sub>-mediated platelet aggregation has been found (Sciulli et al 2006). This is explained by the fact that even tiny concentrations of TXA<sub>2</sub> may activate the platelets. Minuz et al (2006) have shown that low concentrations of TXA<sub>2</sub> activate the tyrosine-kinase-based signaling pathway which may translate into a full platelet activation in the presence of weak platelet agonists or subthreshold concentrations of stronger agonists.

These findings lead to the concept of functional COX-2 selectivity by NSAIDs, ie, inhibition of COX-2 in the presence of an insufficient reduction of platelet COX-1 activity to translate into inhibition of platelet function. It has to be pointed out that most NSAIDs are functionally

selective for COX-2 at therapeutic doses similarly to coxibs. The only tNSAID that has been shown to be functionally non-selective for the platelet is naproxen at high doses, in some individuals (Capone et al 2004). This depends on the fact that naproxen usually administered at high doses bid is more potent for COX-1 than COX-2, and it is characterized by a pharmacokinetic half-life of approx 14 h (Burke et al 2006). Thus, naproxen is associated with profound inhibition of prostacyclin but the possible CV hazard associated with this effect may be mitigated by a parallel profound and persistent suppression of platelet TXA<sub>2</sub> (Capone et al 2004), which translates into a small CV protection or neutral effect (Hernández-Díaz et al 2006; Kearney et al 2006).

Recent findings suggest that for functional non-selective NSAIDs the extent of inhibition of COX-2-dependent prostacyclin may predict the increased incidence of myocardial infarction (MI) (Patrignani et al 2008a). Individual NSAIDs with a degree of COX-2 inhibition  $< 90\%$  at therapeutic concentrations presented a RR of 1.18 (95% CI, 1.02–1.38) while those with a greater COX-2 inhibition had a RR of 1.60 (95% CI, 1.41–1.81).

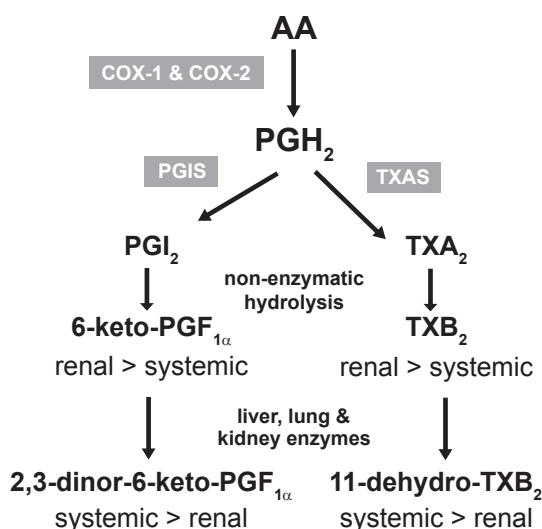
Thus, it has been proposed that the assessment of whole blood COX-2 ex vivo – alone or in combination with the measurement of urinary levels of 2,3-dinor-6-keto-PGF<sub>1 $\alpha$</sub>  [a biomarker of prostacyclin biosynthesis in vivo (Figure 4)] – in association with genetic biomarkers (such as polymorphisms in the prostacyclin receptor IP) (Arehart et al 2008) may be surrogate end-points of CV hazard by pharmacological inhibition of COX-2 (Patrignani et al 2008b).

## Etoricoxib

### Pharmacology

The NSAID selective for COX-2 etoricoxib, 5-chloro-6'-methyl-3-[4-(methylsulfonyl) phenyl]-2,3'-bipyridine (Figure 5) (Riendeau et al 2001) (Arcoxia<sup>®</sup>, Tauxib<sup>®</sup>, Algix<sup>®</sup>) has been approved in Europe, Latin America, and Asia-Pacific region for the treatment of OA, rheumatoid arthritis (RA), ankylosing spondylitis, acute gouty arthritis, acute pain, and chronic musculoskeletal pain, at doses ranging between 60 and 120 mg daily depending on the different indications.

As shown in Figure 6A, etoricoxib is 162-fold more potent towards COX-2 than COX-1 in the whole blood assay in vitro. The pharmacokinetics features of etoricoxib are reported in Figure 6C (Agrawal et al 2003). Importantly, it has a long half-life of 22 h and is metabolized via 6'-methyl hydroxylation in human liver microsomes. The reaction is

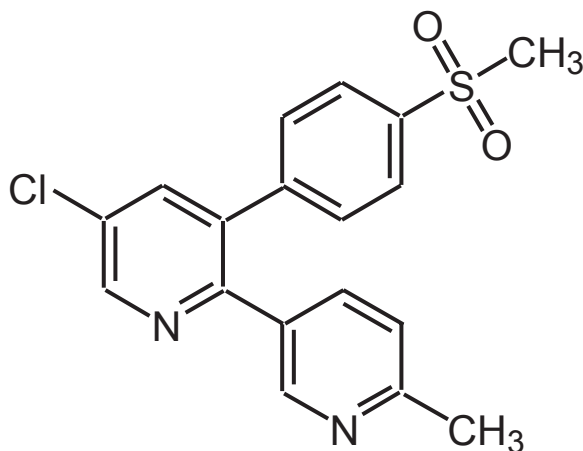


**Figure 4** Biosynthesis and metabolism of prostacyclin ( $\text{PGI}_2$ ) and thromboxane ( $\text{TXA}_2$ ).

**Abbreviations:** PGIS, prostacyclin synthase; TXS, TX synthase.

catalyzed by a number of P450 forms, although CYP3A4 accounts for the majority (40%–90%) of the activity. The remainder of the activity is equally divided between a number of other P450s (eg, CYP2D6, CYP2C9, CYP1A2, and possibly CYP2C19). In this regard, the P450 reaction phenotype of etoricoxib is unique and differs from that of other COX inhibitors (Kassahun et al 2001).

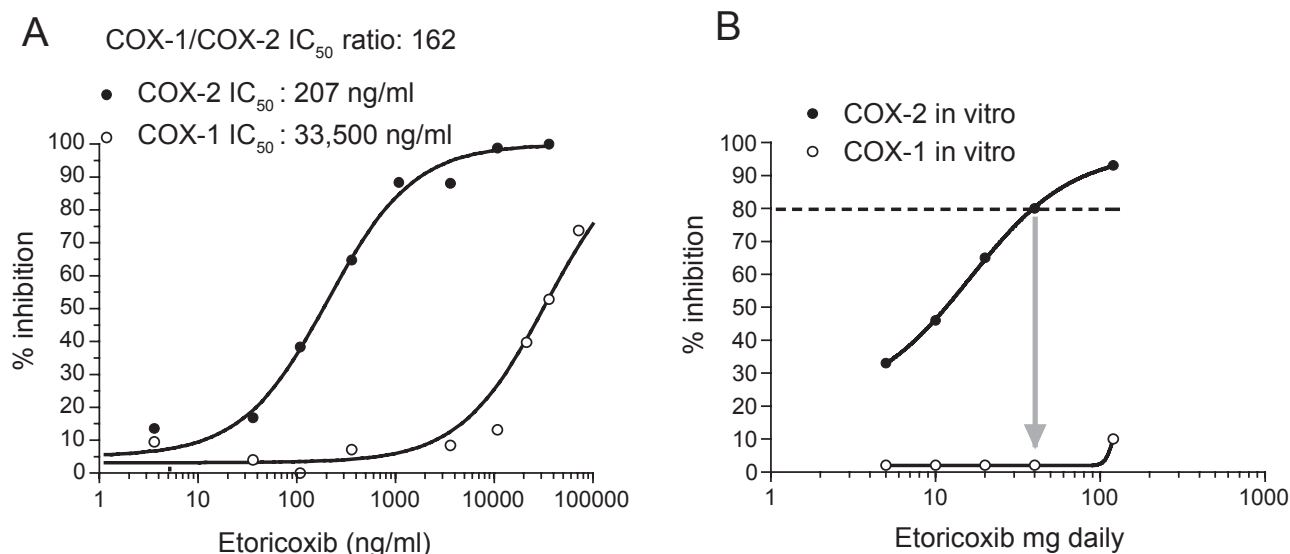
As shown in Figure 6B, etoricoxib administered up to 120 mg is a selective COX-2 inhibitor. This result has been obtained by the extrapolation of COX-1 and COX-2 inhibition *in vitro* in the human whole blood assays by the maximal plasma concentration achieved after the administration of etoricoxib at single doses of 5, 10, 20, 40, 120 mg. Interestingly, an inhibition of COX-2 by 80% may be reached



**Figure 5** Chemical structure of etoricoxib: bipyrindine with a methyl sulfone side chain 5-chloro-6'-methyl-3-[4-(methylsulfonyl) phenyl]-2,3'-bipyrindine.

after the administration of 40 mg, suggesting that the dosage of the drug could be reduced without affecting efficacy.

We performed studies of clinical pharmacology to assess achieved COX-2 selectivity *ex vivo* by the chronic administration of etoricoxib 90 mg daily versus the tNSAIDs diclofenac 75 mg bid, naproxen 500 mg bid, and aspirin 100 mg daily (Figure 7 A–C). As shown in Figure 7A the administration of etoricoxib (at 12 h after dosing), diclofenac (at 4 h after dosing), naproxen (at 12 h after dosing), and low-dose aspirin (at 24 h after dosing) caused a median inhibition of COX-1 of 1%, 60%, 95%, and 98%, respectively. These results show that inhibition of platelet COX-1  $\geq 95\%$  (which translates into inhibition of platelet function) occurs in all subjects treated with low-dose aspirin and in some of those treated with high-dose naproxen, at the interval between doses, sustaining an anti-platelet effect of high-dose naproxen. In contrast, in all subjects treated with diclofenac and etoricoxib, the degree of inhibition of platelet COX-1 was not adequate to affect significantly  $\text{TXA}_2$ -dependent platelet function. However, our results showed a marked inter-subject variability in the inhibition of platelet COX-1 after the administration of diclofenac (range 8–98%) and to a lesser extent also for etoricoxib (range 0%–48%). As shown in Figure 7B, whole blood COX-2 was profoundly affected by etoricoxib, diclofenac, and naproxen, but not by low-dose aspirin (median inhibition and range: 86% [70–90], 98% [58–100], 79% [30–83], and 0% [0–31], respectively). These data show that both diclofenac and etoricoxib were administered at supratherapeutic doses, in fact most of individuals had a COX-2 inhibition higher than 80%, considered sufficient to translate into efficacy. From these values we calculated the achieved selectivity (ie the ratio of COX-2 inhibition and COX-1 inhibition *ex vivo*). As shown in Figure 7C, for etoricoxib and diclofenac the individual values of achieved COX selectivity were placed above 1 (which represents a balanced inhibition of COX-2 and COX-1) showing that circulating concentrations of the two drugs caused a more profound inhibition of COX-2 than COX-1. In contrast, naproxen and aspirin caused a more profound inhibition of COX-1 than COX-2; in fact the ratio of COX-2 /COX-1 inhibition *ex vivo* was always lower than 1. These data clearly show that diclofenac and naproxen, despite being considered tNSAIDs, are significantly different in selectivity compared with COX-isozymes at therapeutic doses. Interestingly, the achieved selectivity was not different, in a statistically significant fashion, between etoricoxib and diclofenac. These results will be relevant for interpretation of the clinical results, for both efficacy and toxicity.

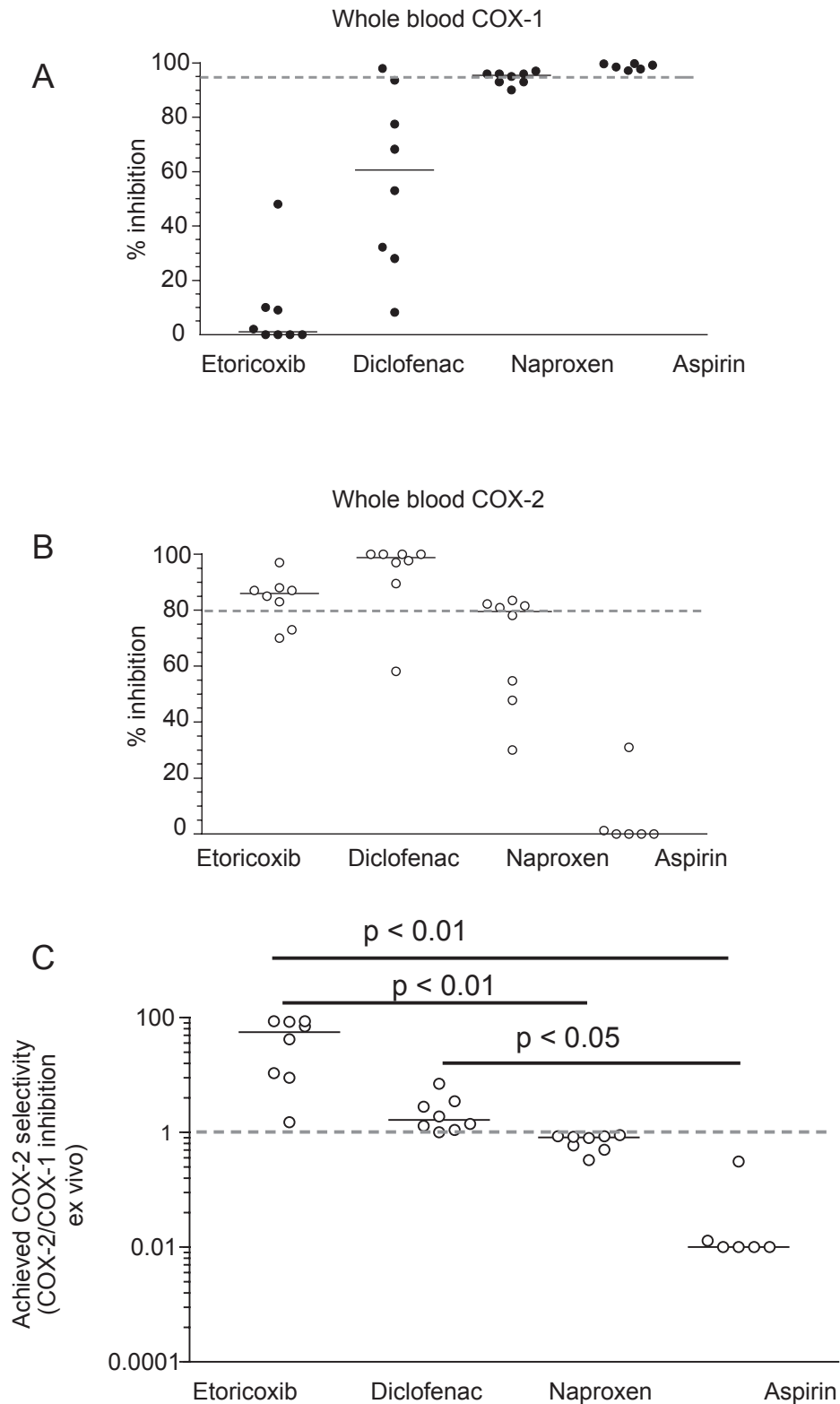


**Figure 6** COX-2 selectivity of etoricoxib in human whole blood. (A) Concentration-response curves assessed in vitro; (B) degree of inhibition of COX-2 and COX-1 by peak plasma concentrations obtained after dosing with etoricoxib 5, 10, 20, 40, and 120 mg; (C) pharmacokinetics features of etoricoxib.

Prostanoids are autocooids that act in the proximity of the cell where they are generated. In fact, they are subjected to an intense metabolism which leads to inactivation of prostanoids. It proceeds through  $\beta$ -oxidation  $\omega$ -oxidation, 15-hydroxy dehydrogenation, and double-bond reduction through enzymatic pathways in liver, kidney, and lung (Ramwell et al 1980; Roberts 1987) (Figure 4). The assessment of major enzymatic

metabolites of prostanoids represents an index of their systemic biosynthesis. Several lines of evidence support a dominant platelet origin (mostly COX-1-derived) of a major urinary enzymatic metabolite of TXB<sub>2</sub>, ie 11-dehydro-TXB<sub>2</sub>, while vasculature (mostly COX-2 derived) represents the dominant origin of a major urinary enzymatic metabolite of prostacyclin, ie, 2,3-dinor-6-keto-PGF<sub>1 $\alpha$</sub>  (Capone et al 2007).



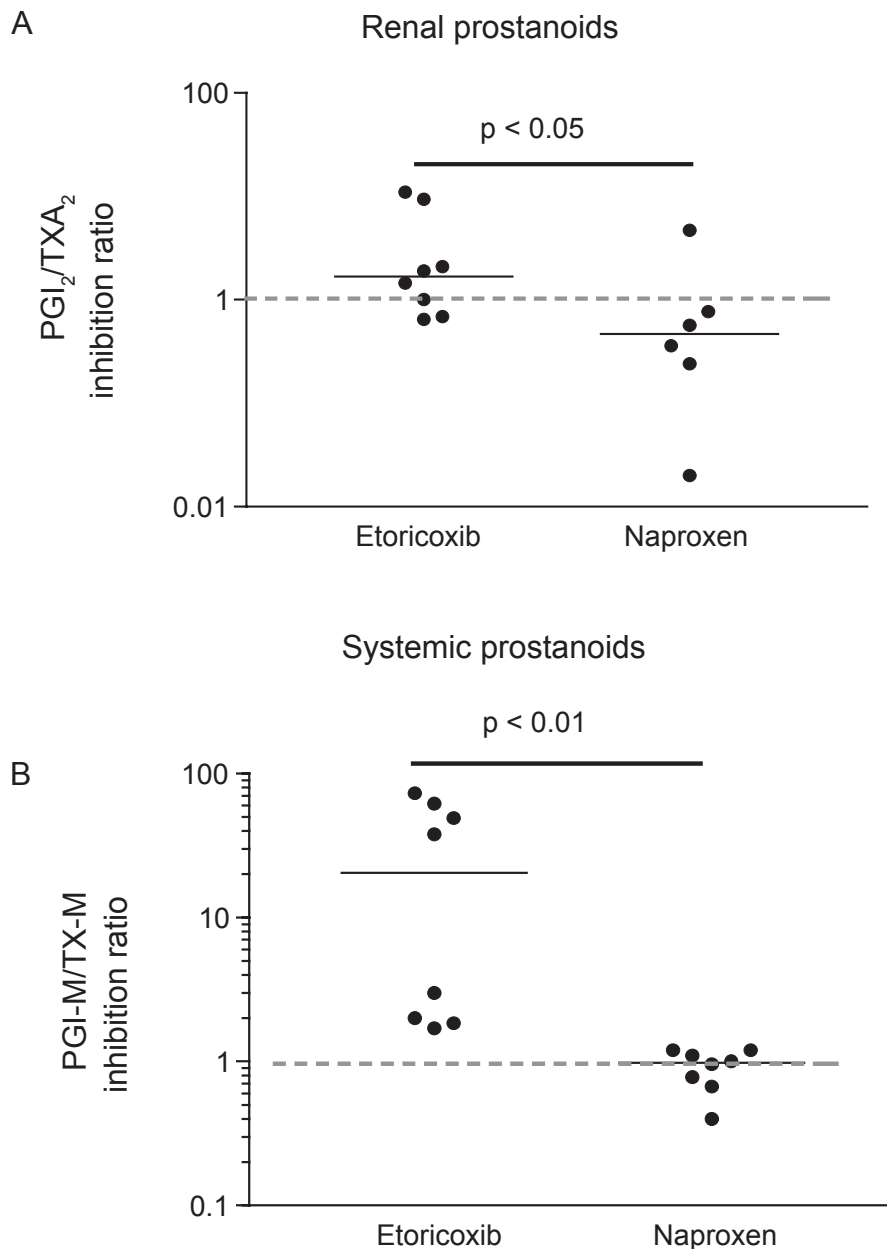


**Figure 7** Whole blood COX-1 (A) and COX-2 (B) inhibition ex vivo after the chronic administration of etoricoxib 90 mg, diclofenac 75 mg bid, naproxen 500 mg bid, and aspirin 100 mg daily to humans; (C) achieved COX-2 selectivity by the drugs.

In susceptible patients, inhibition of prostanoid generation by tNSAIDs and coxibs causes sodium retention with resulting edema and hypertension (Grosser et al 2006). Similarly to thrombogenesis, COX-1 inhibition may mitigate the consequence of COX-2 inhibition in the kidney. This is compatible with a COX-2-dependent source of vasodilatory prostacyclin and COX-1-dependent origin of vasoconstrictors, like TXA<sub>2</sub> in the kidney (Qi et al 2002; Grosser et al 2006). We studied the impact of etoricoxib and naproxen on renal and systemic prostanoid biosynthesis by

assessing 6-keto-PGF<sub>1α</sub>/TXB<sub>2</sub> inhibition ratio and 2,3-dinor-6-keto-PGF<sub>1α</sub>/11-dehydro-TXB<sub>2</sub> inhibition ratio, respectively, after the administration of the drugs (Figure 8).

As shown in Figure 8 A and B, etoricoxib caused a more profound inhibition of COX-2 than COX-1 both at renal and extrarenal sites. This is predictive of a CV hazard but of reduced GI toxicity by etoricoxib. In contrast, naproxen reduced more profoundly COX-1 both at renal and extrarenal sites (Figure 8A and B), which may translate into reduced CV but higher GI side effects.



**Figure 8** Effects of the chronic administration of etoricoxib 90 mg daily and naproxen 500 mg bid to humans on prostacyclin/TXA<sub>2</sub> inhibition ratio in the kidney (by assessing their urinary non-enzymatic metabolites: 6-keto-PGF<sub>1α</sub> and TXB<sub>2</sub>, respectively) (A), versus systemic biosynthesis (by assessing their urinary enzymatic metabolites: PGI-M = 2,3-dinor-6-keto-PGF<sub>1α</sub> [a major urinary enzymatic metabolite of prostacyclin] and TX-M = 11-dehydro-TXB<sub>2</sub> [a major enzymatic metabolite of TXA<sub>2</sub>], respectively) (B).

## Etoricoxib and GI toxicity

The GI safety of etoricoxib has been assessed in the MEDAL (Multinational Etoricoxib vs Diclofenac Arthritis Long Term) Programme (Laine et al 2007). It was prospectively designed to pool data from 3 randomized, double-blind clinical trials: the MEDAL study, the Etoricoxib vs Diclofenac Sodium Gastrointestinal Tolerability and Effectiveness (EDGE) study, and the EDGE II study. Patients with OA or RA aged 50 years or over were eligible for enrollment if they had a clinical diagnosis of OA of the knee, hip, hand, or spine, or a clinical diagnosis of RA that satisfied at least 4 of 7 of the American Rheumatism Association 1987 revised criteria (Arnett et al 1988), and in the judgment of the investigator, would require chronic therapy with an NSAID. Thus, etoricoxib (60 or 90 mg daily) and diclofenac (150 mg daily) were compared in 34,701 patients with OA or RA for upper GI clinical events (bleeding, perforation, obstruction, or ulcer) and the subset of complicated events (perforation, obstruction, witnessed ulcer bleeding, or significant bleeding). As shown in Table 2, there were significantly fewer upper GI clinical events with the COX-2 selective inhibitor etoricoxib than with the tNSAID diclofenac due to a decrease in uncomplicated events, but not in the more serious complicated events. Less inhibition of gastric COX-1 by etoricoxib than diclofenac (etoricoxib is a more COX-2 selective drug than diclofenac *in vitro* and *ex vivo* [Patrignani et al 1997; Tacconelli et al 2002] [Figure 7]) may explain the reduced incidence of uncomplicated GI events. In contrast, the comparable rates of GI bleeding by the two drugs could be due to a similar impact on platelet COX-1, in some individuals, and on healing of gastric lesions. In fact, it has been shown that COX-2 plays an important role in the healing of gastric ulcers and that inhibition of COX-2 delays ulcer healing (Konturek et al 2005). As shown in Figure 7B, etoricoxib and diclofenac have a comparable profound impact on COX-2 (>80%).

In MEDAL, the use of low-dose aspirin and proton pump inhibitors (PPIs) was allowed. Analyzing the data in aspirin and non-aspirin populations, it has been found that etoricoxib reduced the risk of uncomplicated upper GI events compared

with diclofenac in patients taking low-dose aspirin regularly, but the magnitude of the GI benefit decreased with low-dose-aspirin use (aspirin: hazard ratio 0.73, 95% CI 0.64–1.07 versus no-aspirin: 0.52, 0.38–0.71,  $p = 0.021$ ) (Laine et al 2007). PPIs were used concomitantly for at least 75% of the study period by 40% of patients and treatment effects did not differ significantly in these individuals; however, significantly fewer patients discontinued etoricoxib than diclofenac due to dyspepsia, and this decrease was similar in patients who took PPIs, suggesting that the COX-2-selective inhibitor provided symptomatic benefit even in patients already taking a PPI (Laine et al 2007).

## Etoricoxib and CV toxicity

In placebo-controlled RCTs, coxibs (rofecoxib, celecoxib, and valdecoxib) were associated with increased RR of CV events (1.92–3.7) (Ott et al 2003; Bresalier et al 2005; Nussmeier et al 2005; Pfizer 2005; Solomon et al 2005). An overview of data derived from trials with coxibs suggests that MI predominates over stroke (Kearney et al 2006). These results led the regulatory authorities such as the Food and Drug Administration (FDA) and the European Agency for the Evaluation of Medicinal Products (EMA) to restrict the use of these drugs (Patrignani et al 2008c). However, the results of observational studies and a meta-analysis of data derived from trials with coxibs have shown that the CV hazard is also associated with some tNSAIDs, such as diclofenac (Hernandez-Diaz et al 2006; Kearney et al 2006). A plausible mechanism in increased risk of vascular events in individuals treated with NSAIDs selective for COX-2 and some tNSAIDs is the inhibition of COX-2-dependent prostacyclin unaccompanied by inhibition of COX-1-dependent TXA<sub>2</sub> at functional range, ie >95%. In fact, most tNSAIDs are as COX-2 selective as coxibs for platelet function. In addition, coxibs and tNSAIDs inhibit profoundly prostacyclin generation *in vivo*, as assessed by the measurement of urinary levels of a major enzymatic metabolite 2,3-dinor-6-keto-PGF<sub>1 $\alpha$</sub>  (Catella-Lawson et al 1999; McAdam et al 1999).

**Table 2** Risk of GI events with etoricoxib and diclofenac in MEDAL study (derived from Laine et al 2007)

| Type of GI events             | Hazard ratio with etoricoxib vs diclofenac | 95% CI    | p       |
|-------------------------------|--|-----------|---------|
| All upper-GI events           | 0.69                                       | 0.57–0.83 | 0.0001  |
| Uncomplicated upper-GI events | 0.57                                       | 0.45–0.74 | <0.0001 |
| complicated upper-GI events   | 0.91                                       | 0.67–1.24 | 0.0001  |

**Abbreviations:** GI, gastrointestinal; MEDAL, Multinational Etoricoxib vs Diclofenac Arthritis Long Term.

The CV hazard associated with the use of coxibs led to the voluntarily withdrawal from the US and EU market of rofecoxib and valdecoxib, in 2004 and 2005, respectively. Recently, the FDA has formally rejected manufacturer application for approval of etoricoxib as a prescription pain reliever. The FDA decision was influenced by the results of safety analyses from the MEDAL Program (Cannon et al 2006). The authors reported that etoricoxib (60 or 90 mg daily, mean duration of therapy was 18 months) and diclofenac (150 mg daily, ie 50 mg tid or 75 mg bid) administered to OA or RA patients have comparable rates, constant over time, of thrombotic CV events (Cannon et al 2006). Despite etoricoxib and diclofenac having different COX-2 selectivity *in vitro*, they are functional COX-2 selective for platelet function. The shared pharmacodynamic translates into comparable incidence of thrombotic events detected in MEDAL.

Aside from the platelet, the relationship between inhibition of prostaglandin formation and prostaglandin dependent function appears to be roughly linear in other cell types. In this context, even an incomplete inhibition of COX-1 might attenuate the functional effect derived from inhibition of COX-2-dependent prostacyclin. This may occur in the kidney where COX-2 is the source of vasodilatory prostacyclin and COX-1 is the source of vasoconstrictor TXA<sub>2</sub> (Qi et al 2002; Grosser et al 2006). This is sustained by the results of MEDAL where the selective COX-2 inhibitor etoricoxib caused a higher incidence of blood pressure than the less selective tNSAID diclofenac. However, the more pronounced signal from MI rather than stroke showed by the use of NSAIDs (traditional and selective for COX-2) suggests that thrombosis is the dominant component of the CV hazard and thus lesser degrees of selectivity are likely only to mitigate the hazard to a marginal extent.

Both EMEA and FDA and the American Heart Association (Antman et al 2007) issued the recommendations that selective COX-2 inhibitors are contraindicated in patients with ischemic heart disease and/or stroke, that they have to be avoided in patients with risk factors for coronary heart disease, and that all patients have to take the lowest effective dose for the shortest time necessary to control symptoms. Importantly, we have recently shown that the degree of COX-2 inhibition achieved by individual NSAIDs (an index of drug potency/exposure), that do not show functional suppression of platelet COX-1, is a determinant of the CV hazard associated with them (Patrignani et al 2008a, c).

Thus, a possible approach to reduce the CV risk associated with etoricoxib will be to use the lowest effective

dose. Studies of efficacy with lower doses of etoricoxib than those used in MEDAL seem to support this possible strategy. Etoricoxib 30 mg/day has been shown efficacious in OA compared with either ibuprofen 800 mg tid (Wiesenhutter et al 2005) or diclofenac 50 mg tid (Leung et al 2002). Moreover, it has been recently assessed the efficacy of etoricoxib 30 mg daily versus the recommended therapeutic dose of celecoxib, 200 mg daily, in the treatment of OA patients (Bingham et al 2007). The primary hypothesis was that etoricoxib 30 mg would be at least as effective as celecoxib 200 mg for Western Ontario and McMaster (WOMAC) Pain Subscale, WOMAC Physical Function Subscale and Patient Global Assessment of Disease Status. Etoricoxib was non-inferior to celecoxib and both were superior to placebo ( $p < 0.001$ ) for all three outcomes. The authors suggested that etoricoxib 30 mg was at least as effective as celecoxib 200 mg and had similar safety in the treatment of OA of knee and hip. As shown in Figure 6B, etoricoxib 30 mg seems adequate to reduce whole blood COX-2 at a degree appropriate to translate into clinical efficacy. However, further studies of clinical pharmacology in patients with OA and RA should be performed to verify the impact of etoricoxib 30 mg on biochemical markers of COX inhibition, such as whole blood COX-2 and systemic and renal biosynthesis of prostacyclin and TXA<sub>2</sub>.

## Conclusions

NSAIDs are an important and efficacious class of drugs for the management of musculoskeletal symptoms. The development of NSAIDs selective for COX-2 – to reduce the incidence of serious GI adverse effects compared with tNSAIDs mainly dependent on the inhibition of COX-1 in GI tract and platelets – has unravelled the important protective role of COX-2 for the CV system, mainly through the generation of prostacyclin. In a recent nested-case control study, we found that patients taking NSAIDs (both coxibs and tNSAIDs) had a 35% increase risk of MI. The increased incidence of thrombotic events associated with profound inhibition of COX-2-dependent prostacyclin can be mitigated, even if not removed, by a complete suppression of platelet COX-1 activity. In fact, inhibition of TXA<sub>2</sub>-dependent platelet function occurs when platelet COX-1 activity, assessed *ex vivo*, is reduced  $\geq 95\%$  (Reilly and FitzGerald 1987). We have introduced the concept of functional COX-2 selectivity (ie, a profound suppression of COX-2 associated with insufficient inhibition of platelet COX-1 to cause inhibition of platelet function) which is a feature shared by most tNSAIDs and coxibs. We have

recently shown that inhibition of COX-2 and the functional selectivity with which it is achieved is relevant to CV hazard from NSAIDs and relates to drug potency (exposure) (Patrignani et al 2008a, c).

The development of genetic and biochemical markers will help to identify the responders to NSAIDs or who are uniquely susceptible at developing thrombotic or GI events by COX inhibition. This will lead to the development of individual responder approaches. We propose that the assessment of whole blood COX-2 ex vivo (alone or in combination with urinary 2,3-dinor-6-keto-PGF<sub>1 $\alpha$</sub> ) may represent a valid surrogate end-point to predict CV risk for functionally selective COX-2 inhibitors. The use of these biochemical markers together with genetic biomarkers will help to select patients uniquely susceptible to developing CV risk through inhibition of COX-2-dependent-prostacyclin when exposed to NSAIDs. Their use can lead to a rational selection of doses for efficacy and will help to make decisions in risk management of tNSAIDs and NSAIDs selective for COX-2.

## Disclosures

None of the authors has any conflicts of interest to disclose.

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