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Review

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Selective COX-2 Inhibitors and Risk of Myocardial Infarction

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Key Words
Atherothrombosis · Cyclooxygenase · Platelets · NSAID · Cyclooxygenase-2

Abstract
Selective inhibitors of cyclooxygenase-2 (COX-2, 'coxibs') are highly effective anti-inflammatory and analgesic drugs that exert their action by preventing the formation of prostanoids. Recently some coxibs, which were designed to exploit the advantageous effects of non-steroidal anti-inflammatory drugs while evading their side effects, have been reported to increase the risk of myocardial infarction and atherothrombotic events. This has led to the withdrawal of rofecoxib from global markets, and warnings have been issued by drug authorities about similar events during the use of celecoxib or valdecoxib/parecoxib, bringing about questions of an inherent atherothrombotic risk of all coxibs and consequences that should be drawn by health care professionals. These questions need to be addressed in light of the known effects of selective inhibition of COX-2 on the cardiovascular system. Although COX-2, in contrast to the cyclooxygenase-1 (COX-1) isoform, is regarded as an inducible enzyme that only has a role in pathophysiological processes like pain and inflammation, experimental and clinical studies have shown that COX-2 is constitutively expressed in tissues like the kidney or vascular endothelium, where it executes important physiological functions. COX-2-dependent formation of prostanoids not only results in the mediation of pain or inflammatory signals but also in the maintenance of vascular integrity. Especially prostacyclin (PGI_2), which exerts vasodilatory and antiplatelet properties, is formed to a significant extent by COX-2, and its levels are reduced to less than half of normal when COX-2 is inhibited. This review outlines the rationale for the development of selective COX-2 inhibitors and the pathophysiological consequences of selective inhibition of COX-2 with special regard to vasoactive prostaglandins. It describes coxibs that are currently available, evaluates the current knowledge on the risk of atherothrombotic events associated with their intake and critically discusses the consequences that should be drawn from these insights.

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Introduction

The spectacular, worldwide withdrawal of Vioxx (rofecoxib) from the market, followed by a similar warning about Celebrex (celecoxib), has initiated legal and commercial sequelae and has also induced great uncertainty among patients and physicians. Patients experienced a

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loss of confidence in medical professionals and in the pharmaceutical industry, because there was speculation that the atherothrombotic risk of these drugs could have been known before [1, 2]. Among health care professionals, there remains uncertainty especially regarding the question of whether the remaining selective COX-2 inhibitors which are still available on the market hold similar risks, as far less clinical data about these drugs are at hand than for rofecoxib.

Moreover, some new, highly selective COX-2 inhibitors are being launched. To avoid wrongful testing of these drugs, which are highly effective and well appreciated in patients, it will be of critical importance for the pharmaceutical industry in charge as well as for prescribing physicians that these launches will be accompanied by responsible marketing strategies that also inform about potential side effects even if there are no conclusive data from large clinical studies yet.

However, the question of whether the side effects observed for rofecoxib, and more recently also for celecoxib and valdecoxib/parecoxib, represent a class effect of these drugs, or whether this was an effect that was specific for rofecoxib [3], which would not be caused by other coxibs, needs to be examined on the basis of the biological role of cyclooxygenase isoforms, of their products, and of the mode of action of inhibitors of these enzymes.

In the following, we will highlight the physiological role of cyclooxygenases and prostaglandins in vascular biology and shed light on some widespread pathophysiological assumptions that have formed the rationale for the development of selective inhibitors of COX-2. We will then focus on the effectiveness of selective COX-2 inhibitors and draw comparisons between non-specific inhibition of cyclooxygenases and specific inhibition of COX-2. We will outline the pathophysiological consequences of selective inhibition of COX-2 for vascular prostanoid formation and discuss the potential implications of these effects for atherothrombosis according to clinical and experimental knowledge. Finally, we will discuss the relevance of these insights for indications, potential advantages and potential risks of selective COX-2 inhibitors other than rofecoxib that are clinically approved or currently being developed.

Cyclooxygenase Isoforms and Products

The enzyme referred to as cyclooxygenase uses arachidonic acid, which is liberated from membrane-bound phospholipids through phospholipase A_2, as a substrate to generate the endoperoxide prostaglandin G_2 (PGG_2). By the same enzymatic complex, PGG_2 is further biotransformed to another endoperoxide, prostaglandin H_2 (PGH_2) [4–6]. Thus, a more correct terminology refers to this enzymatic complex as prostaglandin H synthase (PGHS), thereby describing an enzyme that possesses two catalytic moieties, that of a cyclooxygenase (generating the endoperoxide structure of PGG_2) and that of a peroxidase (generating PGH_2 from PGG_2), although the widely used description cyclooxygenase usually refers to the whole complex of PGHS [5]. This simplification reflects the fact that many well-known non-steroidal anti-inflammatory drugs (NSAID) inhibit the activity of the enzyme by preventing access of arachidonic acid to the catalytic site of the cyclooxygenase located inside a hydrophobic channel, which is formed by PGHS, without affecting the peroxidase activity, which is located outside this hydrophobic channel [6, 7]. For matters of simplicity, we will retain the biochemically simplified terminology of referring to PGHS as 'cyclooxygenase'.

COX-2 is only one of at least two different isoforms of cyclooxygenase. A recent report even describes a third isoform, 'COX-3', which appears to be a variant of COX-1 expressed in dog brain, and has been discussed to be the target of acetaminophen [8, 9]. As there is no evidence for a role of this isoform, or other yet unidentified COX isoforms in the cardiovascular system, we will confine to the two well-characterized isoforms, COX-1 and COX-2, in this overview.

Following the production of PGH_2, a second enzymatic process is needed to ultimately form the different biologically active prostanoids. This step is not catalyzed by PGHS/cyclooxygenase any more, but by tissue-specific enzymes, which all use PGH_2 as substrates [10]. These enzymes show some specificity with respect to the tissue they are expressed in and also generate specific prostanoid products, which also determine the name of the enzyme. Thus there are prostaglandin I_2 (PGI_2 or prostacyclin) synthase, a thromboxane A_2 (TxA_2) synthase, and prostaglandin D_2, E_2, or F_2 synthases [10]. Like cyclooxygenases, they are widely expressed throughout the human body and have numerous functions in the vascular system (fig. 1 summarizes the biochemical pathways from arachidonic acid liberation to the production of prostanoids).

Inhibition of cyclooxygenases results in decreased substrate availability for such tissue-specific prostanoid synthases and subsequently decreases the production of the specific prostanoid. Little is known as to whether any of the cyclooxygenase isoforms shows preference of associa-

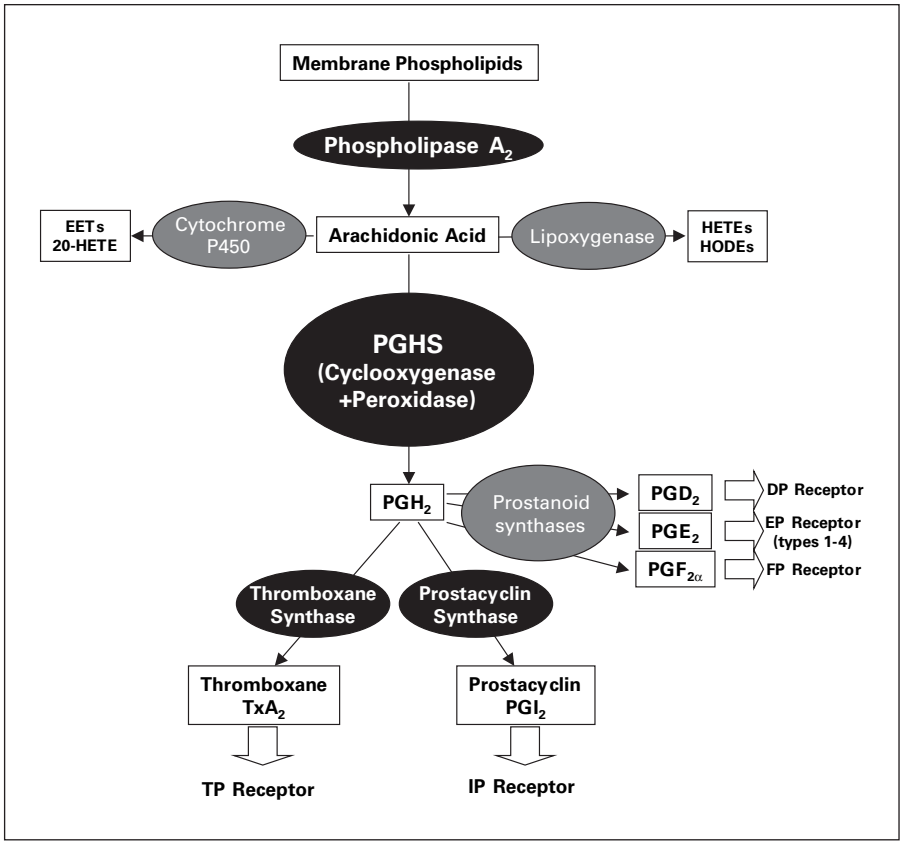

Fig. 1. Biochemical pathway leading from arachidonic acid to the formation of vasoactive prostanoids. Following liberation of arachidonic acid from membrane phospholipids, which is dependent on phospholipase A_2 activity, PGHS competes with cytochrome P_{450} enzymes and lipoxygenase for further metabolism of AA. PGHS contains a cyclooxygenase and peroxidase moiety and produces PGH_2, which needs to be further metabolized by tissue-specific enzymes (prostanoid synthases) to form the specific prostanoids PGI_2, TxA_2, PGD_2, PGE_2 and $PGF_{2\alpha}$. They all act on specific receptors. The most important prostanoids for vascular function are PGI_2 and TxA_2. EETs = Epoxyeicosatrienoic acids; HETEs = hydroxyeicosatetraenoic acids; HODEs = hydroxyoctadecadienoic acids; DP = prostaglandin D_2 receptor; EP = prostaglandin E_2 receptor; IP = prostacyclin receptor; FP = prostaglandin F_2 receptor; TP = prostaglandin T_2 receptor.

tion with any of the downstream prostanoid synthases in general [4, 6, 10]. The association of a cyclooxygenase with a prostanoid synthase, which is decisive for the formation of a specific prostanoid from arachidonic acid, seems to be determined by the tissue of interest and by the specific pathophysiological situation [10]. In platelets for example, which only contain the COX-1 isoform, the major PGH_2-metabolizing isomerase coupled to COX-1 is TxA_2 synthase, which leads to the result that the major arachidonic acid product of cyclooxygenase activity in platelets is TxA_2 [10, 11]. As platelets as well as vascular smooth muscle cells express TxA_2 receptors (TP receptors), the release of TxA_2 from platelets results in platelet aggregation (fig. 2) and to a lesser extent in vasoconstriction [12]. When platelet COX-1 is inhibited by NSAID or by acetylsalicylic acid (aspirin), the resulting inhibition of TxA_2 mediates the desired antiplatelet effect (fig. 2, 3). Thus, aspirin was the first and remains the most important substance counteracting platelet aggregation. However, as NSAID or aspirin do not act directly on TxA_2 synthase, they also inhibit any other cyclooxygenase they reach, independent of the isomerase to which these cyclooxygenases may be coupled. Thus, numerous other physiological effects result because COX-1 or COX-2 enzymes may be inhibited in any tissue. However, some special considerations need to be mentioned with respect to aspirin. First, in contrast to many other NSAID, aspirin binds irreversibly to cyclooxygenase [13]. Second, the dosing of aspirin has an important role, because 'low-dose aspirin' only effectively inhibits platelet cyclooxygenase activity; although a single dose of only 100 mg/day already shows an inhibitory effect on COX-1, it is further increased by repetition of this dose, and low-dose aspirin ultimately blocks TxA_2 synthesis through accumulation in platelets [13]. In nucleate cells, this accumulation would be overcome by rapid novel synthesis of cyclooxygenases, but platelets – being anucleate structures – cannot sufficiently resynthesize cyclooxygenase, so the inhibitory effect of aspirin can only be reversed by novel platelet synthesis from megakaryocytes. In contrast, whereas clinically used doses of other NSAID also have impact on the activity of the enzyme, these drugs do not

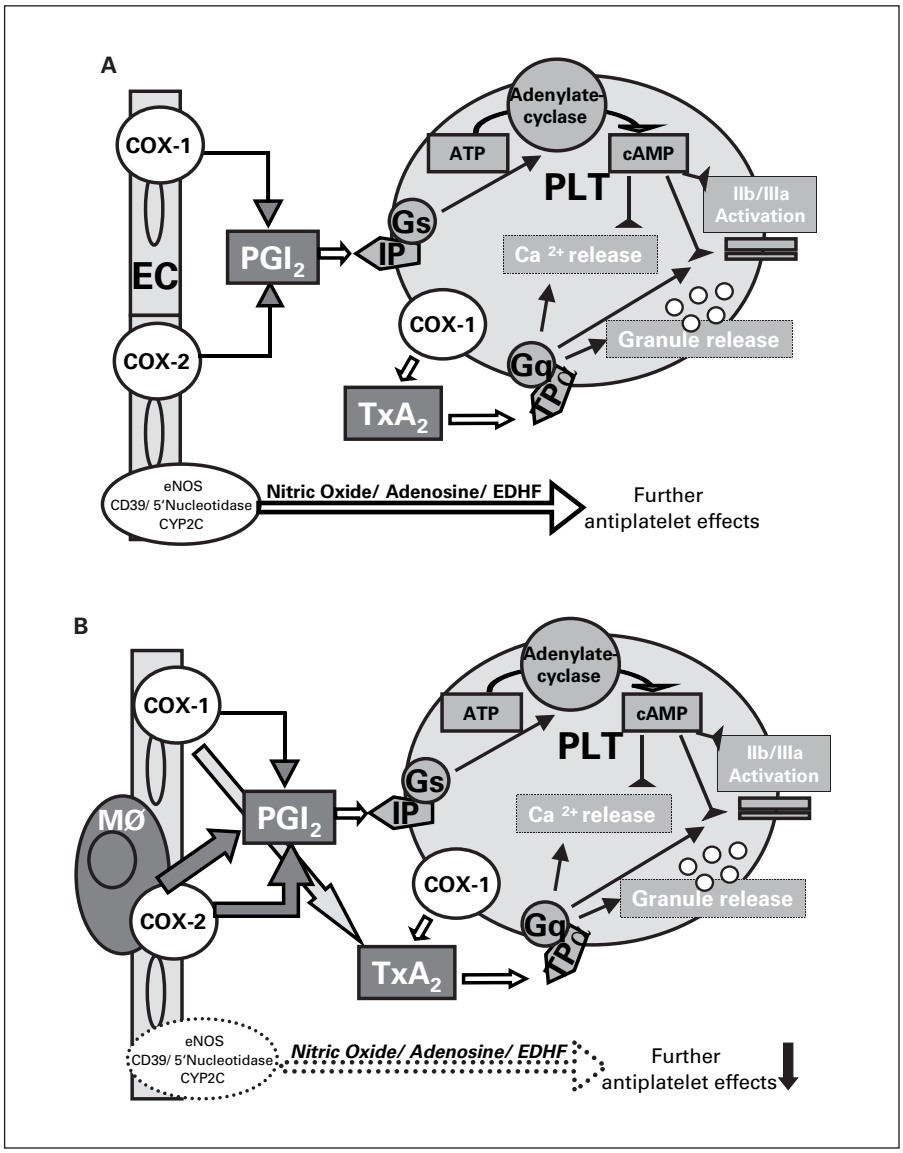

Fig. 2. Effect of platelet-active prostanoids on platelet function. **A** Under physiological conditions, prostanoids influence platelets (PLT) through G-protein-coupled prostanoid receptors. PGI_2 released from the vascular endothelium (EC) activates IP receptors and thus inhibits platelet activation by increasing cAMP levels, which prevents intracellular calcium release and decreases granule secretion and fibrinogen receptor activation (GPIIb/IIIa). The release of PGI_2 from the endothelium depends on both, COX-1 and COX-2. TxA_2 is released mainly by activated platelets and further augments platelet calcium levels, granule secretion and GPIIb/IIIa activation through TP receptors. **B** In atherosclerosis, the situation may change dramatically, because the release of other endothelial autacoids, which decisively contribute to the antiplatelet properties of physiological endothelium, may be decreased. In addition, subendothelial accumulation of macrophages (MØ) may participate in PGI_2 formation, and cyclooxygenases (especially COX-1) may now contribute to vascular levels of TxA_2. EDHF = Endothelium-derived hyperpolarizing factor; IP = prostacyclin receptor; TP = prostaglandin T_2 receptor.

bind irreversibly and usually dissociate from their binding sites at cyclooxygenase [14]. Thus low-dose aspirin only effectively inhibits platelet COX-1 activity (and resulting TxA_2 synthesis), whereas NSAID inhibit all cyclooxygenases (and the resulting formation of other prostanoids), but only do so reversibly.

But why do higher doses of aspirin (or NSAID) not result in more effective inhibition of platelet aggregation? To understand this phenomenon, another vascular cyclooxygenase metabolite with importance for platelet activity comes into focus. This metabolite, prostacyclin (PGI_2), is a potent platelet inhibitor and is formed in intact vascular endothelium through cyclooxygenase coupled to PGI_2 synthase. Whereas repeated delivery of low doses of aspirin has little effect on immediate or long-term cyclooxygenase activity in the endothelium due to the aforementioned transcriptional novel synthesis of cyclooxygenases and because endothelial COX-2 has limited sensitivity to drug [13, 14], high doses of aspirin or NSAID have similar effects on endothelial PGI_2 and platelet TxA_2 synthesis, thus theoretically exerting antithrombotic as well as prothrombotic effects (fig. 3). This circumstance, the limited time span and the reversibility of NSAID binding to cyclooxygenases form the pharmaco-

logical basis for several observations reporting that NSAID are not as effective as low-dose aspirin in inhibiting platelet aggregation [15, 16].

These exemplary considerations are complicated by the fact that cyclooxygenase isoforms are differentially expressed and regulated throughout the vascular system. We have mentioned earlier that platelets only contain the COX-1 isoform. Moreover, COX-1 is expressed almost ubiquitously, and is therefore regarded as a housekeeping enzyme [17, 18], whereas the expression of COX-2 seems to be more regulated. Traditionally, COX-2 is appreciated by most practicing physicians to be a strictly inducible enzyme, which is upregulated upon stimulation with proinflammatory mediators such as cytokines, growth factors, lipopolysaccharides (LPS) or even by prostanoids themselves in cells that participate in inflammatory processes, e.g. macrophages, monocytes or other cells [19, 20]. However, the situation is more complicated, because there is evidence for COX-2 being constitutively expressed in a variety of tissues, and in some of these tissues important physiological functions have been attributed to COX-2-derived prostanoids. Constitutive expression or physiological roles for COX-2 have been described in gastric tissue of rats, rabbits and humans [21, 22], in different functional tissues of human kidney [23], in uterine epithelium [24], human myometrium and fetal membranes [25, 26], in the eye [27] and in the brain [28]. The constitutive expression of COX-2 is of special importance with regard to cells of the vascular system. First, there have been reports about COX-1 being inducible [29, 30], bringing about doubts about the hypothesis of a housekeeping or inducible enzyme. Second, it is now well recognized that COX-2 is constitutively expressed in some cells of the vascular system, e.g. endothelial cells, or cells of the renal medulla, renal vasculature or the macula densa, and participates in the regulation of vessel function through paracrine or autocrine release of certain prostanoids [6, 20]. Moreover, it has been shown that COX-2 constitutively binds to PGI_2 synthase in endothelial cells [31], and, as will be outlined below, numerous data suggest that it is a physiological source of PGI_2 in vivo. Although most prostanoids may be produced by vascular cyclooxygenases under certain conditions and then potentially participate in the regulation of vascular function [5, 32, 33], PGI_2 and the aforementioned TxA_2 (produced mainly from platelets) are the most important prostanoids in the regulation of physiological vascular homeostasis having opposing effects on platelet function.

In addition to different expression patterns of cyclooxygenase isoforms, their specific associations with the

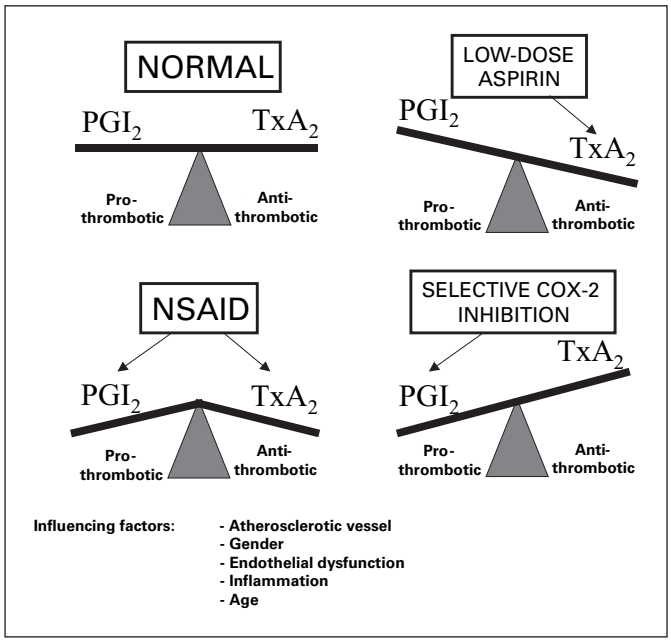

Fig. 3. Influence of low-dose aspirin, non-selective cyclooxygenase inhibition (NSAID, high-dose aspirin) or selective inhibitors of COX-2 on the vascular balance of prostanoids regarding platelet activity and thus thrombosis. The effects of the respective drugs on vascular prostanoid formation and on platelets are depicted schematically. As the in vivo situation is far more complicated, this schematic panel only partly reflects a schematic of a physiological vascular situation. Whereas low-dose aspirin selectively inhibits TxA_2 formation in platelets and thus lowers systemic TxA_2 levels, NSAID (or high-dose aspirin) inhibit cyclooxygenases non-specifically and thus also decrease PGI_2 levels independent of the source. As it is now clear that COX-2 is constitutively expressed in the endothelium and kidney and significantly contributes to systemic PGI_2 formation even in healthy individuals, selective inhibitors of COX-2 decrease systemic levels of PGI_2, without altering TxA_2, resulting in enhanced platelet activation.

PGH_2-metabolizing prostanoid synthases are likely to be of high importance for vascular prostanoid formation, although such specific interaction is only well understood for COX-1 coupling to TxA_2 synthase in platelets.

In summary, in vivo, there is a fine-tuned balance between certain prostanoids produced by specific coupling of cyclooxygenases with tissue-dependent prostanoid synthases which is influenced by the differential expression of cyclooxygenase isoforms. For vascular biology and thrombosis, the resulting effects on platelet-activating TxA_2 and platelet-inhibitory PGI_2 are of crucial importance. Their balance is altered by any drug targeted at cyclooxygenases.

Development, Efficacy and Pharmacology of Selective COX-2 Inhibitors

Because of the assumption that COX-1 is responsible for the production of physiological prostanoids that also mediate cytoprotection in gastric epithelium, whereas COX-2 was supposed to be the strictly inducible isoform mediating inflammation, selective inhibitors of COX-2 were developed. This has led to the assumption that the anti-inflammatory effects of NSAID are solely due to inhibition of prostanoid production by the inducible, 'inflammatory' COX-2, whereas the side effects of these non-specific cyclooxygenase inhibitors are mediated through the inhibition of physiological prostanoid production mediated by COX-1. It appeared logical that selective inhibitors of COX-2 should bring about the advantages of NSAID without their side effects.

Initial experience with rofecoxib and celecoxib – the two selective inhibitors of COX-2 that were developed first – was highly encouraging, as they proved at least one part of the hypothesis by being as effective in fighting inflammation and pain as other NSAID [34–37]. Soon studies were published that aimed at demonstrating that selective inhibitors of COX-2 were also superior to NSAID in terms of gastrointestinal side effects. At first, such evidence was derived from smaller endoscopic studies, which often proved the hypothesis [38, 39]. The first randomized clinical trial showing superiority of a selective COX-2 inhibitor was the VIGOR trial, which compared rofecoxib vs. naproxen for occurrence of gastrointestinal toxicity among 8,076 patients suffering from rheumatoid arthritis [40]. The second large clinical trial designed at proving this superiority of selective COX-2 inhibitors, the CLASS trial, compared celecoxib with diclofenac or ibuprofen in the treatment of arthritis [41]. Although showing similar efficiency, celecoxib failed to prove superior to NSAID in terms of prevention of the primary endpoint, which was overall occurrence of ulcers and erosions, but showed a statistically significant advantage in terms of the secondary endpoint, complicated and symptomatic ulcers [41]. Following the initial success of celecoxib and rofecoxib, which in 2003 accounted for about 75% of sales of the market for NSAID in the US [42], a race for the development of new, even more specific selective inhibitors of COX-2 with improved pharmacokinetics began and led to the production of drugs like valdecoxib (or its prodrug parecoxib for parenteral use), etoricoxib (only approved in some European countries), or lumiracoxib, which was approved in the United Kingdom in September 2004 (the manufacturer has currently halted the application for approval of the European Medicines Agency for European Union countries for lumiracoxib).

These drugs differ not only chemically but also in terms of selectivity for COX-2. Whereas rofecoxib and etoricoxib, both being sulfonyls, show high oral bioavailability and a half-life of 2–3 h (rofecoxib) and 1 h (etoricoxib), celecoxib and valdecoxib (or its prodrug parecoxib) are sulfonamides [6, 43, 44]. Except for valdecoxib, their oral bioavailability is lower than that of the sulfonyls (parecoxib is only available as an intravenous drug), but their half-lives are similar, being 2–4 h [6, 43, 44]. Lumiracoxib, the most recently developed COX-2 inhibitor, has a phenyl acetic acid structure, but also has sufficient oral bioavailability and a half-life of 2–3 h. However, it differs from the previous drugs in terms of selectivity for COX-2 because lumiracoxib is the most selective COX-2 inhibitor known so far [6, 44, 45]. This selectivity of a certain COX-2 inhibitor could be of importance in terms of cardiovascular side effects, as it theoretically should exert direct effects on the balance between TxA_2 and PGI_2. Before clinical application, the selectivity of a drug targeting cyclooxygenases is usually tested by a human whole blood assay. Based on such investigations, rofecoxib and valdecoxib have been found to have comparable selectivities at a COX-1/COX-2 ratio of about 270, which is slightly exceeded by that of etoricoxib, which exhibits a value of about 340. Celecoxib greatly differs from them because its selectivity for COX-2 is rather low when compared to the other coxibs, its COX-1/COX-2 ratio is only about 30 (selectivity data differ markedly according to source, however, the proportions of differences between several coxibs are similar throughout most studies; numbers presented here are taken from a review by FitzGerald [6]). As these data do not necessarily represent the in vivo behavior of a drug, studies corroborating in vivo selectivity of COX-2 inhibitors by showing their omission of an influence on TxA_2 formation have been undertaken and have proved the selectivity of rofecoxib [46], valdecoxib [47, 48], parecoxib, etoricoxib and lumiracoxib [49]. Of note, according to in vitro data, celecoxib is only a little more selective than diclofenac, which also has a ratio in favor of COX-2 of about 20 [6, 14]. This rather low selectivity in comparison to other coxibs may explain the missing advantage of celecoxib in terms of gastrointestinal toxicity, when compared to diclofenac or ibuprofen (which only has a COX-1/COX-2 ratio of about 0.5), and may also be of importance for the cardiovascular side effects that can be expected from a coxib. Except for rofecoxib, which is reduced cytosoli-

cally, all known coxibs are metabolized through oxidation by cytochrome P_{450} enzymes, of which either the 3A4 isoform (celecoxib, valdecoxib/parecoxib or etoricoxib) or the 2C9 isoform (celecoxib, valdecoxib/parecoxib or lumiracoxib) do the job [6, 43, 45, 50]. Metabolism by cytochromes may be of importance for specific side effects of the drugs for two reasons. First, drug-drug interactions with other substances are often due to metabolism by the same cytochrome P_{450} isoform [50]. Such interactions can either lead to an increased plasma level of a drug, when there is competition for metabolism by the same CYP isoform with another drug, or to an increased metabolism through induction of the CYP enzyme, which can cause decreased levels of a drug or even increase the efficiency of a drug if the drug has to be metabolized to be active. A prominent example for the latter is clopidogrel, which is a prodrug metabolized by CYP3A4 to its active form. Second, CYP2C9, especially in the microcirculation, is the source for an important autacoid, the endothelium-derived hyperpolarizing factor [51, 52].

Studies investigating the efficiency of the newer coxibs valdecoxib/parecoxib, etoricoxib and lumiracoxib have all been published and have repeatedly shown the superiority of these drugs in comparison to NSAID in terms of gastrointestinal toxicity [53–55].

Influence of Selective COX-2 Inhibitors on Vascular Formation of Prostanoids

The actual effect of a coxib on prostanoid availability is of high importance for the expected efficiency and the probable side effects of these drugs. In terms of vascular function and thrombosis, the most relevant prostanoids of interest are PGI_2 and TxA_2 [5]. Measurement of cyclooxygenase-derived prostanoids, which are short-lived and cannot be assessed directly, is usually performed through assessment of its metabolites in urine or serum. Thus, serum levels of 6-keto-$PGF_{1\alpha}$ or urine levels of 2,3-dinor 6-keto-$PGF_{1\alpha}$ are usually measured as an index of PGI_2 production, whereas TxA_2 levels are represented by urinary 11-dehydro TxB_2 or serum levels of TxB_2. For matters of simplicity, PGI_2 metabolites will be referred to as PGI-M, whereas TxA_2 metabolites will be referred to as Tx-M in the following.

In an early study comparing rofecoxib with the NSAID indomethacin in elderly adults, it was first observed that COX-2 has a major role in systemic PGI_2 production, because rofecoxib (50 mg/day) was as effective in reducing PGI-M as was indomethacin (50 mg/bid, both grossly halved PGI-M levels after 13 days of treatment), but in contrast to indomethacin, Tx-M left levels unchanged [46]. In a similar study in healthy volunteers, celecoxib, after 4–6 h of dosing only, reduced PGI-M by about 80% (800 mg) or 70% (400 mg), whereas ibuprofen (800 mg) only reached a 66% reduction at that time point [56]. Interestingly, and in accordance with its rather low selectivity for COX-2, one dose of celecoxib (800 mg, assessed at 4 h) also caused a statistically significant reduction of about 28% for one of two Tx-M that were measured (ibuprofen caused a maximum reduction of more than 95% of Tx-M) [56]. Reduction of PGI-M by other selective inhibitors of COX-2 has repeatedly been observed in animal or human models [57–60].

Whereas these data were all obtained in healthy subjects, they do not allow drawing conclusions about the influence of cyclooxygenase inhibitors on prostanoid formation during inflammation or agonist stimulation, where the main prostanoid produced by one cyclooxygenase isoform may not be the same as under basal conditions [32, 61]. The selective inhibitor of COX-2 SC58236 (Pharmacia) blunted the production of PGE_2 and PGI-M in the renal medulla of angiotensin-II-stimulated mice [61]. In a human study comparing the effect of endotoxin (lipopolysaccharide, LPS) on prostanoid metabolism under control conditions, inhibition of COX-1 (low-dose aspirin for 10 days before LPS), non-specific COX inhibition (ibuprofen, 800 mg once prior to LPS), or selective inhibition of COX-2 (celecoxib, 800 mg prior to LPS), both ibuprofen and celecoxib, but not low-dose aspirin, were able to reduce the increase in body temperature caused by LPS. Low-dose aspirin completely reduced platelet-dependent TxA_2 formation, and thus prevented the major part of systemic Tx-M (which also meant that a minor part of systemic TxA_2 was produced in other tissues than platelets), whereas ibuprofen completely blunted Tx-M from any source. Moreover, both ibuprofen and celecoxib drastically reduced levels of PGI-M, whereas low-dose aspirin only had little effect [62]. This showed that COX-2 also significantly contributed to PGI_2 production during endotoxinemia. In atherosclerotic patients, the selective COX-2 inhibitor nimesulide (a rather selective sulfonanilide distributed in some European countries) reduced PGI-M by about 46% [60]. Similar reductions in PGI-M were observed for different selective inhibitors of COX-2 in hypertension [63], upon stimulation with norepinephrine [64], under hypoxic conditions [65] or in LDL-receptor knockout mice [66], whereas Tx-M levels remained unchanged [63, 66] or were even elevated [65]. There is evidence that the vascular expression

of COX-2 may even be upregulated with age or in advanced atherosclerosis. The effect of COX-2 inhibition on both TxA_2 and PGI_2 release was more pronounced in aortic rings from aged rats compared to younger animals [67]. Similar observations, such as a beneficial effect of COX-2 inhibition on flow-mediated dilation in male patients with atherosclerosis, have stimulated a discussion about potential beneficial effects of COX-2 inhibition in the treatment of cardiovascular disease [68, 69]. However, other studies in human [70] or animal models [71] of endothelial function, atherosclerosis or inflammation failed to observe such beneficial effects.

In addition to factors like age, the stage of atherosclerotic diseases or the extent of preexisting endothelial function, gender and the interaction of prostanoids with the production of other autacoids contribute to the role of COX-2-dependent prostanoid formation in vivo. In ovariectomized mice deficient in endothelial NO synthase, estrogen treatment increased prostanoid-dependent vasodilatation, but failed to do so in the presence of functional NO production [72]. Estrogen has recently been shown to upregulate COX-2-dependent PGI_2 production in female mice [73] concomitant with decreased oxidant stress and platelet activation. Another study observed increased renal PGE_2 and TxA_2 production associated with greater medullary COX-2 expression in female spontaneously hypertensive rats [74]. In this study, however, orchidectomy led to an increase in PGE_2 release in males, indicating that male sex hormones might also contribute to gender-dependent differences in prostanoid production [74].

Altogether, the data at hand so far suggest that in contrast to NSAID or low-dose aspirin, selective inhibitors of COX-2 usually have no effect on systemic TxA_2 levels, but rather reduce PGI_2 release in healthy individuals. Although inflammatory diseases or disorders of the cardiovascular system, e.g. hypertension, may have altered cyclooxygenase isoform expression patterns or altered production of cyclooxygenase-dependent prostanoids, the effect of coxibs on PGI_2 levels seems to prevail.

Risk of Arterial Thrombosis

Given that PGI_2 and TxA_2 are the two most important prostanoids with respect to platelet activation, it is easy to perceive that any drug that selectively reduces plasma levels of a physiological antiplatelet substance like PGI_2, without altering levels of the corresponding platelet activator, TxA_2, theoretically has an intrinsic likeliness of increasing the activity of circulating platelets. However, although selective COX-2 inhibitors have been suspected to increase the risk for intravital platelet activation and subsequent thrombosis due to clinical findings or theoretical considerations ever since the VIGOR trial, initial experimental approaches aimed at proving this hypothesis did not succeed in finding indications for an enhanced risk of thrombosis, which may have been due to the low sensitivity of the assays used (platelet deposition in histological samples) [59]. First evidence for an enhanced thrombotic risk under elective inhibition of COX-2 was gathered by Hennan et al. [75] in dogs: in this study, high-dose aspirin had no effect on coronary artery thrombotic occlusion unless it was withdrawn and a recovery time for the endothelium to resynthesize cyclooxygenase was allowed for. After the endothelium had recovered cyclooxygenase (but not platelets because of the irreversible binding of aspirin), there was an increased time to thrombotic occlusion, but this antithrombotic effect was prevented by the administration of celecoxib during recovery. Very recently, two experimental studies, using either highly sensitive methods for assessing enhanced thrombogenicity or more adequate disease models, clearly proved that there is a thrombotic risk under selective inhibition of COX-2 in vivo. The first of these studies, which was published just few days before the withdrawal of Vioxx from global markets, used a highly sensitive in vivo microcirculatory model. It revealed that selective inhibition of COX-2 enhanced platelet activation, leading to increased platelet rolling at the intact arteriolar wall. Moreover, firm platelet adhesion was increased and ultimately a markedly reduced time to thrombotic occlusion upon vessel wall damage resulted [57]. The second study – appearing shortly thereafter – showed that during hypoxia in the pulmonary circulation of rats, there was enhanced platelet activation under selective inhibition of COX-2 [65]. Notably, in all these studies, selective inhibitors of COX-2 have not been reported to cause spontaneous thrombosis [57, 59, 65]. Nevertheless, these studies could prove what already was theoretically plausible: that selective COX-2 inhibitors enhance platelet activation and thus are able to trigger the onset of thrombotic events.

Although the VIGOR study had not been designed to investigate side effects of rofecoxib, it brought about the alarming result of a nearly 5-fold increased risk of myocardial infarction in those patients that received rofecoxib; they would have needed to take low-dose aspirin for secondary cardiovascular prophylaxis, but could not do so because of the study design [40]. This finding has

Table 1. List of studies showing an increased risk of atherothrombotic events during intake of selective inhibitors of COX-2

Coxib	Study or authors	Patients	Publication date	Reference No.
Rofecoxib	VIGOR	8,076	2000	40
	APPROVe	2,586	2004	78
	Kaiser-Permanente	~1.4×10^6	2004	www.fda.gov/cder/drug/infopage/vioxx/vioxxgraham.pdf
	Juni et al.	20,742	2004	79
	Solomon et al.	54,475	2004	80
Celecoxib	APC	2,035	2004	82
Parecoxib/ valdecoxib	McSPI (CABG)	462	2003	85
	Nussmeier et al.	1,671	2005	86

Of note, in some of the studies shown here, an increased risk for rofecoxib but not for celecoxib was observed. One analysis even found an increased risk during intake of NSAID (Kaiser-Permanente). CABG = Coronary artery bypass grafting; APC = Adenoma Prevention with Celecoxib Trial; APPROVe = Adenomatous Poly Prevention on Vioxx Trial.

prompted vigorous discussion among scientists as to whether this was a mechanistically induced 'class' effect of all selective COX-2 inhibitors, or whether it was an intrinsic problem of rofecoxib. Alternatively, it was discussed whether the increase in myocardial infarctions in the rofecoxib group was due to an intrinsic antiplatelet – and thus cardioprotective – property of naproxen, which was used as the NSAID that rofecoxib was compared with. The authors of the VIGOR study related this difference to such a potential antiplatelet property of naproxen. However, future studies failed to convincingly prove its cardioprotective properties [16, 76, 77]. Naproxen – being an NSAID – turned indeed out to be able to inhibit platelet COX-1-dependent TxA_2 production almost as effectively as low-dose aspirin, which is why it can prevent platelet aggregation in ex vivo assays (where there is no endothelium to supply PGI_2), but also inhibited systemic PGI_2 production in vivo, which is a critical difference when comparing it to low-dose aspirin [16]. In our opinion, naproxen is unlikely to have exerted an aspirin-like coronary-protective effect in the VIGOR study. In addition, it has recently become clearer that rofecoxib may indeed have intrinsic atherothrombotic features. A correlation between myocardial infarction and rofecoxib was also found in the recent APPROVe study, which was stopped because there was an increased risk of atherothrombotic complications after 18 months of rofecoxib intake [78]. Large meta-analyses of randomized trials yielded similar results (table 1) [79, 80]. Although these circumstances had prompted the withdrawal of rofecoxib from global markets, many questions remained. Besides ethical questions relating to market introduction of a new drug, to date the most important question for clinicians is whether all the other coxibs also exert a prothrombotic effect and if so, whether only long-term use or short-term intake of a coxib bears the risk of myocardial infarction?

There are more data at hand now to shed light on the question of a potential class effect: the manufacturer of *celecoxib* has issued a warning of potential cardiovascular atherothrombotic side effects in December 2004 due to preliminary results from the Adenoma Prevention with Celecoxib Trial [81], which gives evidence for dose- and time-of-intake-related increases in cardiovascular events due to celecoxib [82]. Before this, there had been one incidental report about thrombotic events in 4 patients with connective tissue disease who had taken celecoxib [15], but neither the large randomized CLASS study nor any clinical trial had shown an enhanced risk of myocardial infarction following celecoxib treatment, and some surveys that observed an enhanced risk for rofecoxib failed to do so in patients taking celecoxib (table 1) [80, 83]. This lack of prior evidence may have been due to the fact that the CLASS study was conducted in a cohort of patients who – in contrast to the study population in VIGOR (rheumatoid arthritis) – did not have an increased risk for cardiovascular disease (mainly osteoarthritis patients) [84]. However, the lower COX-2 selectivity of celecoxib, which also partly reduces Tx-M levels in vivo [56], gives reason to question whether celecoxib will really cause an

enhanced atherothrombotic risk. In support of a theory assigning higher risks of adverse cardiovascular events to those coxibs that are more specific are recent data from a study of parecoxib/valdecoxib in patients undergoing coronary artery bypass grafting [85]. The most recently published study by Nussmeier et al. [86] confirmed these initial observations in a larger population. However, a retrospective meta-analysis screening nearly 8,000 patients who had received valdecoxib at different dosing regimes during clinical studies for osteoarthritis or rheumatoid arthritis found no increased incidence of myocardial infarction when comparing valdecoxib with nonselective NSAID [87]. The recent TARGET trial comparing lumiracoxib, which is the most selective COX-2 inhibitor so far, with naproxen and ibuprofen also found no correlation between the incidence of myocardial infarction and treatment with lumiracoxib [88]. To the best of our knowledge, no data in support of such as risk for lumiracoxib or etoricoxib are at hand up to date, but several smaller studies did not find an increased risk of myocardial infarction during their use [55, 89]. Table 1 summarizes the clinical evidence for atherothrombotic events associated with different coxibs.

Although theoretically the risk of atherothrombotic events should increase with the selectivity of the drug for COX-2, this property of a coxib alone does not suffice for the event to occur with a statistically significant likeliness. Other factors, such as the dose of the drug that is taken and the time of intake, will naturally contribute to the likeliness of an event to occur. In addition, there is good evidence that the prothrombotic risk is most pronounced in patients who are already at an increased risk of atherothrombotic events due to their underlying disease.

Other Cardiovascular Effects

Because of their likeliness to selectively inhibit endothelial PGI_2 synthesis and thus unbalance the equilibrium between vascular TxA_2 and PGI_2 in favor of the vasoconstrictor TxA_2, several other cardiovascular or related effects, e.g. disturbance of vision (due to potentially altered blood supply) [90], may theoretically occur. Like NSAID, coxibs are likely to moderately elevate systemic blood pressure [91], which is probably due not only to detrimental effects on the endothelial function but also to nephrotoxicity [92]. However, the effect of selective inhibition of COX-2 on endothelial function may also be beneficial, as repeatedly shown in patients with ischemic heart disease [68, 69]. These findings are in accordance with a proinflammatory role of COX-2-derived PGI_2 in atherosclerosis development [66] and have formed a basis for discussing a potential use of selective COX-2 inhibitors in patients with coronary heart disease [93]. Similar to myocardial infarction, further atherothrombotic effects such as stroke or pulmonary embolism are likely to occur at increased rate, and this has already been observed in some of the clinical studies [78, 86]. The APPROVe trial also reported differences in groups in events like congestive heart failure, pulmonary edema or cardiac failure [78]. However, the likeliness for an adverse event to occur may differ markedly according to the underlying disease, as prostacyclin synthesis is also known to interact with nitric oxide synthesis in the vasculature, with diverging net outcomes for thrombosis, endothelial function or atherogenesis.

Outlook

According to the data available on selective COX-2 inhibitors and their known effects on vascular pathophysiology and the balance between PGI_2 and TxA_2, it is highly likely that the risk for thrombotic events is a class effect inherent in coxibs. This effect is likely to occur preferentially in patients who already have an increased risk to experience atherothrombosis, because selective inhibitors of COX-2 do not cause thrombosis themselves, but rather support its onset. For some time, partly because of a lack of sufficient data, the risk had only been suspected for rofecoxib, but newer data suggest that it may also complicate the use of other coxibs. In addition to the physiological mechanisms leading to the described vascular imbalance between PGI_2 and TxA_2, other vascular phenomena, such as the recently described upregulation of thrombomodulin by COX-2-formed prostanoids [94] or the diverse interactions of endothelial autacoids, e.g. NO, with prostanoid production [95], may also contribute to the enhanced risk of thrombotic complications. Due to pharmacokinetic and chemical differences in the various substances available, some of these compounds may not bear this risk at all. Thus, a critical evaluation as to the extent of this risk should be performed for each of the COX-2 inhibitors separately. Such evaluation should compare the extent of in vivo inhibition of PGI_2 synthesis with TxA_2, as the balance between these prostanoids is the key to an effect of any cyclooxygenase inhibition on thrombosis and can easily be assessed in humans. Moreover, the pathophysiology of the underlying diseases of individual patients destined to be treated with coxibs

must be considered when using these drugs. Whereas some patient groups may benefit from their advantages, others are more likely to be harmed by detrimental effects, as nicely demonstrated in a statistical post hoc analysis of the VIGOR population [96]. It should be borne in mind that selective COX-2 inhibitors are well tolerated by most patients, that they offer reliable relief from pain and inflammation, and that they are highly appreciated by those who use them. All health professionals should be aware of the fact that there is a risk. However, they should also be aware of its likely limitations and thus be able to individually decide on patients who are apt to receive selective COX-2 inhibitors. If the cardiovascular risk of a certain patient is in doubt, they should be prescribed cautiously. In the present modern medicine, physicians face increasing numbers of patients with complex, multiple disease. It is not unlikely that a patient may require effective anti-inflammatory treatment but also has a high risk for both gastrointestinal bleeding and cardiovascular thrombosis. In these patients, physicians may want to consider the concurrent use of selective COX-2 inhibitors with antiplatelet agents other than low-dose aspirin, who do not have gastrointestinal toxic side effects, and corresponding studies may be warranted for special patient subsets.

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