Thromboxane Receptors Antagonists and/or Synthase Inhibitors

Giovanni Davì, Francesca Santilli, and Natale Vazzana

Contents

G. Davì (✉) • F. Santilli • N. Vazzana
Internal Medicine, University of Chieti, Chieti, Italy
e-mail: gdavi@unich.it

P. Gresele et al. (eds.), *Antiplatelet Agents*, Handbook of Experimental Pharmacology 210, DOI 10.1007/978-3-642-29423-5_11,

Abstract Atherothrombosis is the major cause of mortality and morbidity in Western countries. Several clinical conditions are characterized by increased incidence of cardiovascular events and enhanced thromboxane (TX)-dependent platelet activation. Enhanced TX generation may be explained by mechanisms relatively insensitive to aspirin. More potent drugs possibly overcoming aspirin efficacy may be desirable. Thromboxane synthase inhibitors (TXSI) and thromboxane receptor antagonists (TXRA) have the potential to prove more effective than aspirin due to their different mechanism of action along the pathway of TXA_2. TXSI prevent the conversion of PGH_2 to TXA_2, reducing TXA_2 synthesis mainly in platelets, whereas TXRA block the downstream consequences of TXA_2 receptors (TP) activation.

TXA_2 is a potent inducer of platelet activation through its interaction with TP on platelets. TP are activated not only by TXA_2, but also by prostaglandin (PG) D_2, PGE_2, $PGF_{2\alpha}$, PGH_2, PG endoperoxides (i.e., 20-HETE), and isoprostanes, all representing aspirin-insensitive mechanisms of TP activation. Moreover, TP are also expressed on several cell types such as macrophages or monocytes, and vascular endothelial cells, and exert antiatherosclerotic, antivasoconstrictive, and antithrombotic effects, depending on the cellular target.

Thus, targeting TP receptor, a common downstream pathway for both platelet and extraplatelet TXA_2 as well as for endoperoxides and isoprostanes, may be a useful antiatherosclerotic and a more powerful antithrombotic intervention in clinical settings, such as diabetes mellitus, characterized by persistently enhanced thromboxane (TX)-dependent platelet activation through isoprostane formation and low-grade inflammation, leading to extraplatelet sources of TXA_2. Among TXRA, terutroban is an orally active drug in clinical development for use in secondary prevention of thrombotic events in cardiovascular disease. Despite great expectations on this drug supported by a large body of preclinical and clinical evidence and pathophysiological rationale, the PERFORM trial failed to demonstrate the superiority of terutroban over aspirin in secondary prevention of cerebrovascular and cardiovascular events among ~20,000 patients with stroke. However, the clinical setting and the design of the study in which the drug has been challenged may explain, at least in part, this unexpected finding.

Drugs with dual action, such as dual TXS inhibitors/TP antagonist and dual COXIB/TP antagonists are currently in clinical development. The theoretical rationale for their benefit and the ongoing clinical studies are herein discussed.

Keywords Antiplatelet therapy • Atherothrombosis • Ischemic stroke • Platelet activation • Thromboxane biosynthesis • TP antagonists

1 Introduction

Atherosclerosis and its clinical manifestations (i.e., ischemic heart disease, cerebrovascular or peripheral artery disease) are major causes of mortality and morbidity in Western countries.

Conventional antiplatelet agents such as aspirin or clopidogrel are currently used in the prevention of cardiovascular events. However, more effective drugs with less bleeding or gastrointestinal complications are desirable.

Thromboxane (TX) A_2 is involved in a diverse range of physiological and pathophysiological processes, including thrombosis, asthma, myocardial infarction (MI), inflammation, acquired immunity, and atherogenesis. Thus, the stimulation of TX/endoperoxide receptors (TP) elicits diverse biological effects under both normal and pathological conditions. Stimulation of TP results in activation of different signaling cascades that regulate the cytoskeleton, cell adhesion, motility, nuclear transcription factors, proliferation, cell survival, and apoptosis (Nakahata 2008).

TXA_2 is the major product of the arachidonic acid (AA) metabolism in platelets that, in response to various stimuli, is produced via the consequent actions of cyclooxygenase (COX) and TX synthase (TXS). Through its interaction with TP receptors on platelets, TXA_2 is a potent inducer of platelet activation (Davì and Patrono 2007).

Furthermore, the activation of endothelial TP promotes the expression of adhesion molecules and favors adhesion and infiltration of monocytes/macrophages. TP receptors exhibit a wide distribution within the cardiovascular system; in fact, these receptors are membrane-bound G protein-coupled receptors (GPCR) found not only on platelets but also on circulating inflammatory cells, such as macrophages or monocytes, and in vascular endothelial cells, and smooth muscle cells (Meja et al. 1997; Miggin and Kinsella 1998).

Thus, antagonists of TP may have some advantages over aspirin as they not only block the effect of TXA_2 on platelets, but also inhibit other ligands such as prostaglandin (PG) endoperoxides and isoprostanes. Because of the wide distribution of TP receptors in platelets, in the vascular wall or in atherosclerotic plaques, TP antagonists inhibit also the effects of TXA_2 over TP receptors on vascular cells or in the plaque. Therefore, antagonists of TP receptors may not only have antiplatelet effects but also impact endothelial dysfunction and the inflammatory component of atherosclerosis (Chamorro 2009).

2 TP Receptors and Their Antagonism

2.1 Molecular and Cellular Biology

TP receptor is highly distributed in various tissues. In addition to platelets, endothelial cells, smooth muscle cells, glomerular mesangial cells, cardiac myocytes, and many other cells express the TP receptor (Fig. 1) (Nakahata 2008).

The multiple cellular signaling and regulatory mechanisms activated through TP have been only recently characterized. In particular, it has been reported that reactive oxidant species (ROS)-dependent upregulation of TP expression may increase TXA_2/isoprostane responses. This mechanism may significantly contribute

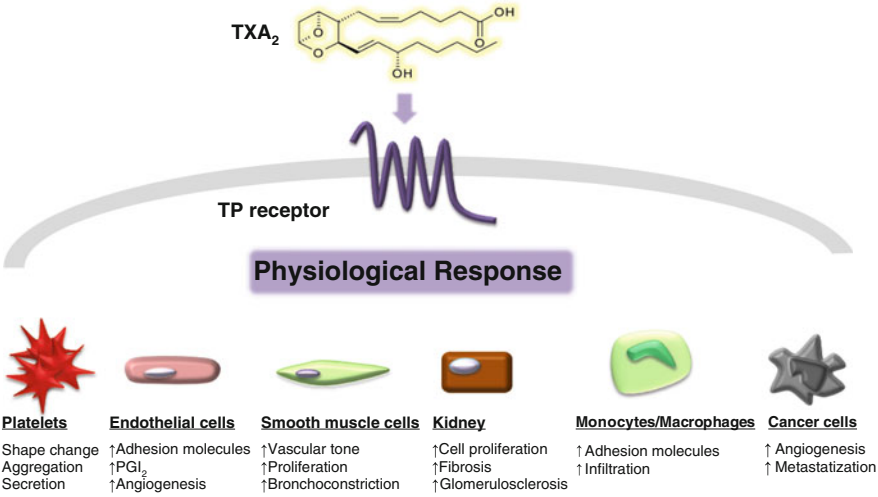

Fig. 1 TP receptor expression and functions. The thromboxane (TX) A_2 receptor (TP) is expressed in a variety of tissues and cells such as platelets, endothelial cells, vascular and bronchial smooth muscle cells, kidney, monocyte/macrophages, and cancer cells. TP activation in these cells is critically involved in the pathobiology of a wide range of diseases, including atherothrombosis, hypertension, renal disease, immune/inflammatory disease, and cancerogenesis. Abbreviations: *PGI_2* prostacyclin

to platelet activation and atherothrombosis in several clinical setting associated with enhanced oxidative stress, such as hypercholesterolemia, obesity, and type 2 diabetes mellitus (T2DM). In addition, posttranscriptional modifications of the receptor, such as phosphorylation or glycation, also determine TP internalization or ligand desensitization. Whether a TP antagonist may affect this receptorial cross-talk and these regulatory pathways is still unanswered.

TXA_2 is the major product of the AA metabolism in platelets that, in response to various stimuli, is produced via the consequent actions of COX and TXS (Davì and Patrono 2007). Low-dose aspirin inhibits platelet TXA_2 production through permanent inactivation of the COX activity of the enzyme prostaglandin G/H-synthase (Fig. 2), and it has clearly been shown to be cardioprotective in several clinical settings (Patrono et al. 2005b). However, many nucleated cells and tissues produce TXA_2 in response to proinflammatory signals and oxidative stress, which acts in an autocrine or paracrine manner as aspirin-insensitive TP agonists. TP receptors are activated not only by TXA_2, but also by PGD_2, PGE_2, $PGF_{2\alpha}$, PGH_2, PG endoperoxides (i.e., 20-HETE), and isoprostanes (Fig. 2).

By binding to TP receptor, these molecules activate several signaling cascades which regulate endothelial cell activation (i.e., adhesion molecules expression), vascular smooth muscle cell (VSMC) contraction, and platelet aggregation, thereby accelerating progression of atherosclerotic lesions (Dogne et al. 2005) (Fig. 3).

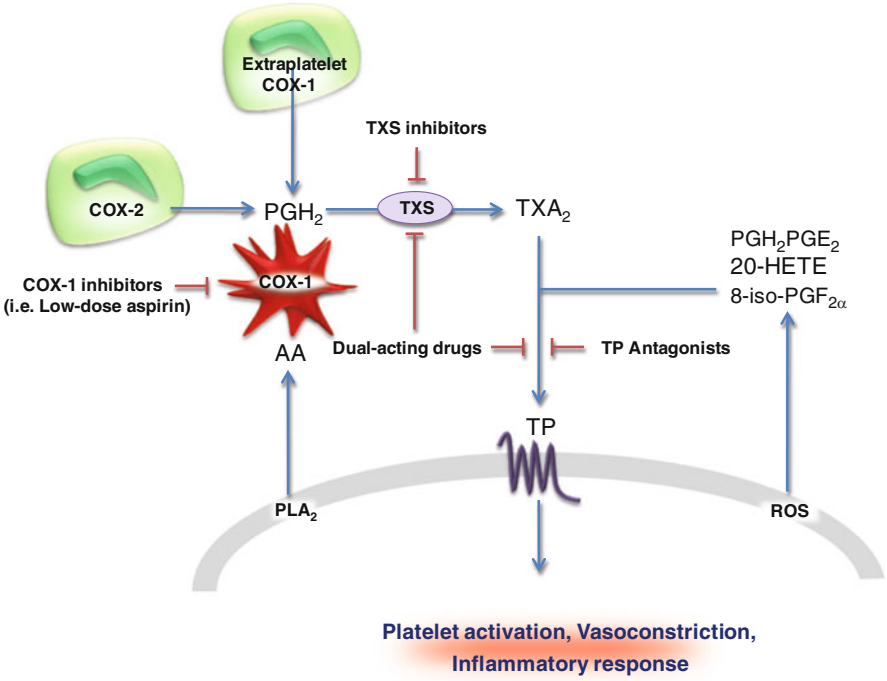

Fig. 2 Acting sites of drugs affecting the TXA_2/TP pathway. Low-dose aspirin is an irreversible inhibitor of the cyclooxygenase (COX) activity of platelet prostaglandin (PG) H_2 synthase-1. Thromboxane synthase (TXS) inhibitors (i.e., ozagrel) block the conversion of PGH_2 to TXA_2. However, TXS antagonism may increase PGH_2, which acts on TP as an agonist, thereby counteracting the antithrombotic efficacy. Dual-acting drugs (TXS inhibitors/TP antagonists: ridogrel, picotamide) and TP antagonists (terutroban) act downstream by blocking the activation of the thromboxane receptor (TP) by TXA_2 as well as other prostanoids, including PGH_2, PGE_2, endoperoxides, and F2-isoprostane. Abbreviations: *HETE* hydroxyeicosatetraenoic acid

In addition to platelets and endothelial cells, TP receptors are also expressed in other cell types involved in atherothrombosis, such as VSMCs (Miggin and Kinsella 1998), macrophages, and monocytes (Meja et al. 1997) (Fig. 1). More recently, it has been reported that TP is expressed in prostate cancer where it regulates cell motility and cytoskeleton reorganization, thus representing a novel target of antineoplastic interventions.

2.2 *TP Receptor Signaling in Platelets*

TXA_2 positively amplify platelet aggregation and is responsible for platelet aggregation in the presence of low concentrations of aggregatory stimuli, such as epinephrine and thrombin. This effect is fully reversed by aspirin or selective antibodies blocking TXS (Mehta et al. 1986).

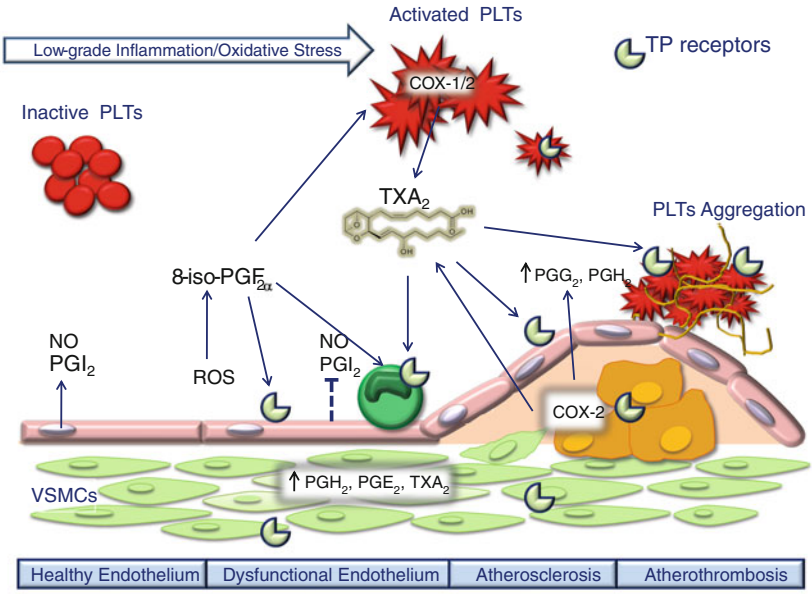

Fig. 3 The role of TXA_2/TP receptor pathway in atherothrombosis. Within the atherosclerotic plaque, the TP receptors are widely expressed on platelets (PLTs), endothelial cells, vascular smooth muscle cells (VSMCs), circulating monocytes, and resident macrophages. Stimulation of TP receptors by their ligands (TXA_2, prostaglandins, endoperoxides, and F2-isoprostanes) activates several signaling cascades which regulate endothelial cell activation (i.e., adhesion molecules expression), VSMCs contraction, and platelet aggregation, thereby accelerating progression of atherosclerotic lesions. Abbreviation: NO nitric oxide

Both TXA_2 and its precursor PGH_2 bind the TP receptor in platelets. TXA_2 is more potent than PGH_2 in initiating aggregation in platelet-rich plasma with EC50 of 66 ± 15 nM and 2.5 ± 1.3 mM, respectively. Conversely in washed platelets, PGH_2 is more potent than TXA_2 with EC50 values of 45 ± 2 and 163 ± 21 nM, respectively. However, whether PGH_2 may significantly contribute to the responses attributed to TXA_2 in vivo is still to be investigated (Mayeux et al. 1988).

In washed human platelets, $PGF_{2\alpha}$ and PGD_2 display lower affinity interactions to TP receptor (Mayeux et al. 1988). Individual prostanoid receptors display ~20–30% sequence identity and encode specific motifs common only to members of the subfamily of PG receptors. Given their structural similarities, PGs may activate more than one subtype of PG receptor. F2 isoprostanes are nonenzymatic, free radical-catalyzed products of AA relatively more stable than TXA_2. These molecules, in particular 8-iso-$PGF_{2\alpha}$, have been shown to bind TP and modulate the adhesive reactions and activation of platelets induced by low levels of other agonists.

2.3 TP Receptor Signaling in Vascular Endothelial and Smooth Muscle Cells

Increased vascular tone due to generation of prostanoids is a main feature of endothelial dysfunction. In fact, endothelium dysfunction is characterized by an increased production of prostanoids (i.e., TXA_2), which facilitate the penetration of macrophages in the vessel wall (Feletou et al. 2009). On endothelial cells, TXA_2 activates the expression of adhesion proteins, such as ICAM-1, VCAM-1, and endothelial leukocyte adhesion molecule-1 (ELAM-1) (Ishizuka et al. 1996). TP-receptor dependent expression of ICAM-1, VCAM-1, and ELAM-1 is mediated by protein kinase C (Ishizuka et al. 1998). TP activation also stimulates the expression of leukocytes adhesion molecules (LAM) on endothelial cells (Ashton et al. 2003). TP activation also promotes prostacyclin (PGI_2) production from endothelial cells through a negative feedback counterregulatory response (Cheng et al. 2002). In fact, PGI_2 attenuates platelet aggregation and VSMC contraction.

In several cardiovascular diseases, endothelial dysfunction is the result of the release of endothelium-derived contracting factors (EDCF) that counteract the vasodilator effect of nitric oxide (NO) produced by the endothelial NO synthase. These endogenous TP agonists produced by the vascular endothelium cause endothelium-dependent contraction and contribute to endothelial dysfunction, a key factor in atherogenesis. Endothelium-dependent contractions involve activation of COXs, production of ROS along with that of EDCFs, which diffuse toward the vascular smooth muscle cells and activate their TP.

Besides the activity of endothelial COX-1, the activation of TP-receptors on VSMCs is also relevant (Swinnen et al. 2009). TP-receptor activation stimulates VSMC proliferation and hypertrophy (Uehara et al. 1988), by potentiating the mitogenic effects of platelet derived growth factor (PDGF) and by increasing the synthesis and release of endogenous basic fibroblast growth factor (bFGF) (Ali et al. 1993).

3 Drugs Affecting TXA_2 Action: Other than COX Inhibitors

The dramatic success story of aspirin as antiplatelet drug had a paradoxically negative effect on the development of drugs that work by closely related but distinct mechanisms to that of aspirin (Fig. 2). TXS inhibitors and TP antagonists were perceived as too close to aspirin to compete effectively with this inexpensive and effective drug.

3.1 Inhibitors of Thromboxane Synthase

The inhibitors of TXS prevent the conversion of PGH_2 to TXA_2. These drugs reduce TXA_2 synthesis mainly in platelets and may improve TXA_2-mediated

pathophysiological conditions, such as thrombosis formation and thrombosis-related disorders (Dogne et al. 2004). TXS inhibitors also enhance vascular generation of PGI_2, which prevents platelet aggregation induced by all known agonists (FitzGerald et al. 1985). In fact, TXS inhibition leads to accumulation of PG endoperoxides in platelets that may be donated to endothelial prostacyclin synthase at sites of platelet–vascular interactions (endoperoxide "steal") (FitzGerald et al. 1985). Consistent with this hypothesis, increased PGI_2 generation in vivo has been reported after administration of several TXS inhibitors (FitzGerald et al. 1985). As PGI_2 may inhibit platelet activation by both TX-dependent and TX-independent mechanisms, it has been proposed that TXS inhibitors may be more effective than aspirin to prevent atherothrombosis.

Several TXS inhibitors—including dazoxiben, dazmagrel, pirmagrel, ozagrel, isbogrel, and furegrelate—have been tested in clinical settings associated with enhanced TX generation.

The greatest experience in human thrombotic disease has been gained with dazoxiben. However, the benefits of this molecule in patients with coronary heart disease were limited or absent (FitzGerald et al. 1985; Kiff et al. 1983; Thaulow et al. 1984).

Ozagrel has been used clinically since 1992 in Japan for the treatment of asthma. Treatment with ozagrel significantly reduced TX generation in patients with coronary or cerebrovascular disease (Uyama et al. 1985; Yui et al. 1984). However, this effect resulted into limited clinical benefit (Shikano et al. 1987).

It has been proposed that incomplete suppression of TX generation in vivo may partly account for the lack of clinical efficacy of these drugs. In addition, TXS inhibition increases PGI_2 generation as well as the formation of other prostanoids, including PGH_2 and endoperoxides, which act as TP agonists, thereby counteracting the reduction of TXA_2-mediated events (Nakahata 2008).

3.2 Dual TXS Inhibition/TP Antagonism

Combined TXS inhibitors and TP antagonist may theoretically overcome the limitations observed for TXS inhibitors. In fact, these drugs do not affect (or enhance) PGI_2 generation, while preventing TP activation by residual TX as well as by other agonists (Patscheke 1990).

Accordingly, the combined administration of a dual TXS inhibitor/TP antagonist gives stronger inhibition of platelet aggregation and prolongs bleeding time more than either drug alone or acetylsalicylic acid (Patrono 1990).

Ridogrel is a drug developed more than 20 years ago as a more potent antiplatelet agent than aspirin. Ridogrel is a TXA_2 inhibitor with additional TP antagonist properties that further enhance its antiaggregatory effects by diverting endoperoxide intermediates into the PGI_2 production pathway (Meadows and Bhatt 2007).

Ridogrel has been studied primarily as an adjunctive agent to thrombolytic therapy in acute MI. In 1993, animal studies showed that ridogrel limits MI size after mechanical coronary occlusion and reperfusion at doses enhancing coronary thrombolysis by streptokinase (Meadows and Bhatt 2007; Vandeplassche et al. 1993).

Thus, the Ridogrel Versus Aspirin Patency Trial (RAPT) was performed to compare the efficacy and safety of ridogrel with that of aspirin as conjunctive therapy for thrombolysis in patients with acute MI. However, despite positive results from initial pilot studies, the largest clinical study, the RAPT, failed to demonstrate any advantage with this agent over aspirin. In fact, in the study of 907 patients with acute MI, there was no difference in the primary end point of infarct vessel patency rate between those randomized to ridogrel (72.2%) or aspirin (75.5%). Despite ridogrel was not superior to aspirin in enhancing the fibrinolytic efficacy of streptokinase, it was more effective in preventing new ischemic events (The Ridogrel Versus Aspirin Patency Trial 1994).

In ulcerative colitis, local production of PGE_2, PGI_2, and TXA_2 has been demonstrated. The inflammatory infiltrate in ulcerative colitis consists of polymorphonuclear leukocytes, mononuclear leukocytes, and macrophages, all of which release considerable amounts of TXA_2. Although PGE_2 may have protective effects on intestinal mucosa, TXA_2 appears to promote the development of chronic inflammatory lesions in the bowel. Thus, an imbalance between the synthesis of cytoprotective prostaglandins, such as PGE_2, and of the pro-inflammatory TXA_2 may play a role in the development of chronic inflammation and mucosal damage in patients with ulcerative colitis.

Treatment with selective inhibitors of TXA_2 synthesis, including ridogrel, reduced the release of TXA_2, tissue damage, and the development of chronic inflammatory lesions in the colon. A pilot clinical trial in patients with chronic ulcerative colitis demonstrated that high-dose ridogrel (300 mg twice daily) significantly reduced colonic mucosal TXA_2 release, to 31% of basal levels, without significantly reducing the levels of protective PGE_2. However, two multicentre, randomized, double-blind studies failed to find significant differences in the primary efficacy outcome measure among two different doses of ridogrel and placebo. Thus, there was no clear indication in either trial of an effective dose of ridogrel in the treatment of ulcerative colitis (Tytgat et al. 2002).

Various mechanisms are likely responsible for the results observed with ridogrel in clinical trials, including potentially ineffective TP inhibition with the concentrations of ridogrel used in human studies. As such, there currently are no clinical indications for preferential use of ridogrel over aspirin.

Another drug with dual action (TXS inhibition/TP antagonism) is picotamide. In a double blind, randomized trial (ADEP), 2,304 patients with intermittent claudication were allocated to receive picotamide or placebo. However, the trial showed only a nonsignificant benefit of picotamide versus placebo in patients with peripheral artery disease (PAD) (Basili et al. 2010; Neri Serneri et al. 2004).

More recently, picotamide has been studied in diabetics with PAD randomized to receive picotamide or aspirin for 2 years (DAVID study). Overall mortality, the

predefined primary end point, was significantly lower among patients who received picotamide (3.0%) than in those who received aspirin (5.5%) with a relative risk ratio for picotamide versus aspirin of 0.55 (95% CI 0.31–0.98%). Conversely, the combined end point of mortality and morbidity has a slightly lower incidence in the picotamide group, but this difference does not reach statistical significance (Neri Serneri et al. 2004). The results of this study should be cautiously interpreted in the light of its limited statistical power and sample size. In fact, as confirmed by a recent meta-analysis comparing the efficacy of different antiplatelet treatments in patients with PAD, picotamide, like aspirin, is not associated with a statistically significant reduction in cardiovascular adverse events (Violi and Hiatt 2007). Conversely, a significant reduction in cardiovascular risk is observed with thienopyridines, suggesting that the presence of PAD may render platelet activation more critically dependent on ADP than on TXA_2 release.

4 Dual COXIB/TP Antagonists: A Possible New Twist in NSAID Pharmacology and Cardiovascular Risk

In the early 1990s, a new class of nonsteroidal anti-inflammatory drug (NSAID) became available (COX-2 inhibitors, or COXIBs). The gastrointestinal safety, dependent upon lack of COX-1 inhibition, coupled with the emerging evidence of cardiovascular hazard associated with COXIBs, leading to withdrawal of rofecoxib and valdecoxib, suggested that a potentially safer pharmacological approach should be combining the anti-inflammatory activity of COXIBs together with a cardioprotective component which might involve antagonism of TP receptors. This could be achieved by making a simple combination of existing drugs targeted against COX-2 or the TP receptor.

The possibility to combine powerful anti-inflammatory activity with TP antagonism within a single chemical entity provides the basis for a novel class of safer NSAIDs and to plan highly innovative studies of structure–activity relationships, chemical syntheses, and pharmacological investigations.

It has recently been demonstrated (Selg et al. 2007) that a traditional NSAID (diclofenac) and a selective COXIB (lumiracoxib) possess an additional activity: weak competitive antagonism at the TP receptor (Rovati et al. 2010). However, in light of the importance of maintaining a fine balance between TP receptor antagonism activity and COX-2 inhibition, the co-administration of two different molecules is not the best approach because it may result in significantly different pharmacokinetic profiles. Combining both activities into a single chemical entity represents a far better strategy (Selg et al. 2007): in fact, developing a compound with a more “balanced” pharmacological profile relative to these two activities may help evaluating if blocking the activity of TP might counterbalance the deleterious cardiovascular effects driven by the PGI_2 inhibition observed for COX-2 inhibitors.

4.1 TP Antagonists

Several TP antagonists have been developed since the early 1980s. Development of the earliest TP antagonists has been stopped because of their toxicity (or moderate activity) in clinical situations, whereas others have not been investigated for cardiovascular indication, and only the more recent TP antagonists reached clinical evaluation for their antithrombotic properties (Dogne et al. 2004).

Thus, many TP antagonists have been developed for the treatment of TP-mediated diseases, such as ifetroban, sulotroban, daltroban, linotroban, ramatoroban, and seratrodast. Among them, seratorodast is an orally active TP antagonist used clinically for the treatment of asthma in Japan since 1997 (Nakahata 2008).

However, evidence has been accumulated for a competitive TP antagonist, terutroban (S-18886), as a potential candidate for atherothrombosis treatment, for blocking atherosclerosis progression, and for transforming lesions towards a more stable phenotype.

Terutroban is an orally active TP antagonist in clinical development for use in secondary prevention of thrombotic events in cardiovascular disease. Terutroban has been developed as a highly specific, high-affinity TP antagonist. Binding studies show that the drug displaces the binding of [3H]-SQ29548 on human platelet membranes with a Ki value of 0.65 nmol/L, and the Kd value for binding of [3H]-S18886 to human platelet membranes averaged 0.96 nmol/L (Zuccollo et al. 2005). Unlike aspirin and clopidogrel that bind their respective receptor in an irreversible manner, terutroban has a reversible and dose-dependent antithrombotic effect within 96 h (Gaussem et al. 2005).

Escalating doses of terutroban (30 and 100 μg/kg/day) compared with that of aspirin (5 mg/kg/day) and clopidogrel (3 mg/kg/day) show a significant dose-dependent effect on platelet aggregation. When used at higher doses, terutroban is able to reduce collagen-dependent platelet aggregation, at least as well as clopidogrel. Terutroban reduces platelet deposition on low shear (212 s^{-1}) and high shear (1,690 s^{-1}) rate conditions (platelet thrombus formation in the Badimon perfusion chamber) at least as well as clopidogrel, whereas aspirin does not have any significant effect on platelet deposition. Thus, TP antagonists might be useful in clinical settings characterized by severe arterial injury and high shear rate, such as acute coronary syndromes and during and immediately after percutaneous transluminal coronary angioplasty (Chamorro 2009).

Pharmacokinetics and pharmacodynamics of terutroban have been studied in patients with PAD (Gaussem et al. 2005). Peak plasma levels are reached between 30 min and 2 h and the half life is 5.8–10 h. Maximal inhibition of U46619-induced platelet aggregation is achieved within 1 h, and this effect is maintained for at least 12 h. Over the range of studied doses, there is a predictable relation between plasma drug concentration and degree of platelet inhibition. Plasma concentrations above 10 ng/mL strongly inhibit U46619-induced platelet aggregation. These plasma concentrations are reached only by dosages higher than 10 mg/day (Gaussem et al. 2005).

The antithrombotic effects of increasing doses (1–30 mg/day) of terutroban have also been demonstrated in PAD (TAIPAD study) using a design based on the ex vivo evaluation of platelet aggregation. This effect was predictable, dose-dependent with maximal inhibition at 1 h, and lasted for approximately 48 h at the oral dose of 30 mg (Fiessinger et al. 2010).

Terutroban does not bind other prostanoid receptors, such as IP or DP receptors, and thus preserves antivasoconstrictive effects of their natural ligands, PGI_2 and PGD_2 (Chamorro 2009).

5 Pathophysiological Rationale for the Superiority of TP-Receptor Antagonists Over Aspirin

5.1 Advantages as an Antithrombotic Agent

An increased incidence of cardiovascular events and enhanced TX-dependent platelet activation characterize several clinical conditions, and aspirin is thought to be the best choice in these settings. However, enhanced TX generation may be explained by several mechanisms relatively insensitive to aspirin.

Monocytes and macrophages are the largest source of TXA_2 and are capable of newly synthesizing TXA_2 via their COX-2 pathway, which has a higher threshold of inhibition by aspirin than platelet COX-1. Thus, extraplatelet, nucleate sources of TXA_2 biosynthesis, possibly triggered by inflammatory stimuli, are less affected than platelet TXA_2 production by the once-daily regimen of administration and by the low dose administered, and may be an additional reason for the less than expected response to aspirin.

Moreover, in clinical settings characterized by enhanced platelet generation, younger reticulated platelets are increased, platelet COX-2 expression is up-regulated, and a consistent TX production may be driven by this enzymatic pathway relatively insensitive to aspirin (Guthikonda et al. 2007; Santilli et al. 2009).

Moreover, TP antagonists block all TP agonists; these include not only TXA_2 but also endoperoxide (i.e., 20-HETE) and several isoprostanes, nonenzymatic products of fatty acid oxidation formed under conditions of increased oxidative stress (Davì and Falco 2005) and which are not inhibited by aspirin (Fig. 2). Aspirin has no effect on isoprostanes and can actually increase endoperoxide (i.e., HETE) production by COX (Meade et al. 1993). Recently, it has been reported that the signaling mechanism of flow-induced constriction of human and rat cerebral arteries involves enhanced production of ROS, COX activity, and is mediated by 20-HETE via TP receptors (Toth et al. 2011).

Oxidative stress is responsible for enhanced peroxidation of AA to form biologically active F2-isoprostanes, such as 8-iso-$PGF_{2\alpha}$, that is widely recognized as a reliable marker of lipid peroxidation both in vitro and in vivo (Davì and Falco 2005; Patrono et al. 2005a).

Concentrations of 8-iso-$PGF_{2\alpha}$ in the range of 1 nmol/L to 1 μmol/L induce a dose-dependent increase in platelet shape change, calcium release from intracellular stores, and inositol phosphates (Patrono et al. 2005a). Moreover, 8-iso-$PGF_{2\alpha}$ causes dose-dependent, irreversible platelet aggregation in the presence of low concentrations of collagen, ADP, AA, and PGH_2/TXA_2 analogs that, when acting alone, fail to aggregate platelets (Patrono et al. 2005a). These effects are prevented by TP receptor antagonists and 8-iso-$PGF_{2\alpha}$ may cross-desensitize biochemical and functional responses to TX mimetics. These properties may be relevant to settings where platelet activation and enhanced free-radical formation coincide, such as in T2DM.

Patients with T2DM have a two- to fourfold increase in the risk of coronary artery disease, and patients with T2DM but without previous MI carry the same level of risk for subsequent acute coronary events as nondiabetic patients with previous MI (Schramm et al. 2008). Diabetic patients exhibit platelet "hyperreactivity" both in vitro and in vivo (Ferroni et al. 2004), (Davì et al. 1990) enhanced platelet regeneration (Watala et al. 2005), coupled with biochemical evidence of persistently enhanced TX-dependent platelet activation (Ferroni et al. 2004). Urinary TXA_2 metabolites, reflecting the whole biosynthesis of TXA_2 in the body by platelet and extraplatelet sources, are significantly higher in diabetes, with the absolute postaspirin values of 11-dehydro-TXB_2 in diabetics being comparable to nonaspirinated controls (Davì et al. 1990; Ferroni et al. 2004) and such residual TXA_2 biosynthesis despite low-dose aspirin treatment has been shown to be predictive of vascular events in high-risk settings, including diabetes (Eikelboom et al. 2002). Thus, elevated urinary 11-dehydro-TXB_2 levels identify patients who are relatively resistant to aspirin and who may benefit from alternative antiplatelet therapies or treatments that more effectively block in vivo TXA_2 production or activity.

Aspirin remains the cornerstone of antiplatelet prophylaxis, but appears to have limited benefit in diabetes (Baigent et al. 2009). TP antagonists, blocking the interaction of both aspirin-sensitive and aspirin-insensitive agonists should theoretically provide more potent protection against the anticipated detrimental effects of platelet activation (Fig. 4).

The hypothesis that hyperglycemia-induced oxidant stress in T2DM might trigger enhanced generation of 8-iso-$PGF_{2\alpha}$ and that this compound might, in turn, contribute to platelet activation is supported by the finding that 8-iso-$PGF_{2\alpha}$ formation is correlated with the rate of TXA_2 biosynthesis in this setting (Davì et al. 1999) and that intensive antidiabetic treatment is associated with a reduction in both urinary 8-iso-$PGF_{2\alpha}$ and TX metabolites excretion rates (Patrono et al. 2005a).

Thus, both COX-2-derived TXA_2 and F2-isoprostanes may act as aspirin-insensitive agonists of the platelet TP receptor, accounting for the less than complete inhibition of platelet aggregation in T2DM.

Another mechanism advocated in favor of TP antagonism, stands in its capacity to leave COX-1 and COX-2 or any pathway of prostanoid synthesis unaltered, thus preserving the cardioprotective eicosanoid PGI_2, an important homeostatic mechanism of endothelial thromboresistance triggered by platelet activation.

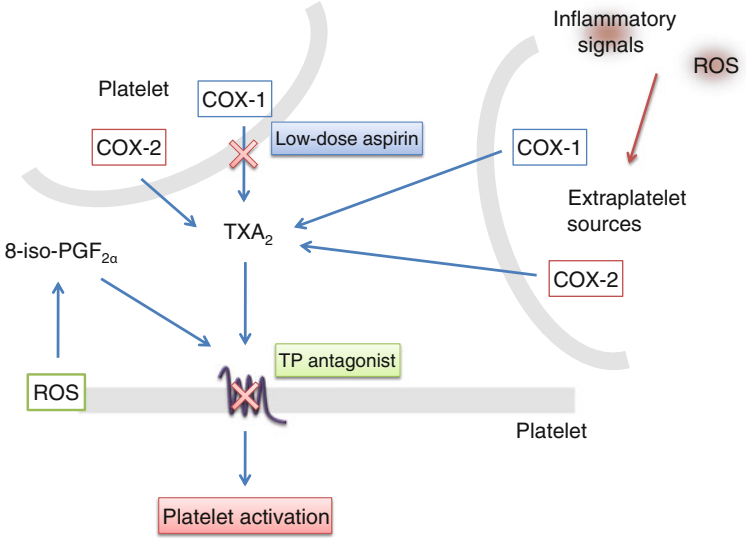

Fig. 4 Possible advantages of TP antagonism over aspirin. Low-dose aspirin irreversibly blocks TXA_2 generation by platelet COX-1. However, in high-risk patients, accelerated platelet turnover may increase platelet COX-1 recovery of TXA_2 biosynthesis as well as COX-2-generated TXA_2. In addition, enhanced oxidative stress and chronic inflammation further stimulated TXA_2 generation by extraplatelet COX-1 and COX-2. Finally, lipid peroxidation by reactive oxidant species (ROS) is able to generate biologically active F2-isoprostane. These molecules, in particular 8-iso-$PGF_{2\alpha}$, have been shown to bind TP and modulate the adhesive reactions and activation of platelets induced by low levels of other agonists. By blocking TP activation by TXA_2 and other aspirin-insensitive agonists, TP antagonists should theoretically provide more potent protection against the detrimental effects of platelet activation

In contrast, depression of COX-2-derived PGI_2 by traditional NSAIDS or COXIBs as well as by anti-inflammatory doses of aspirin removes a constraint on platelet COX-1-derived TXA_2 and other agonists that elevate blood pressure, promotes atherogenesis, and augments the thrombotic response to plaque rupture.

5.2 *Relevance of TP Receptors Inhibition in Atherosclerotic Disease*

Treatment with a TP antagonist, but not treatment with aspirin, inhibits atherogenesis in apo-E deficient mice (Cayatte et al. 2000), strongly suggesting that TP antagonists could be superior to aspirin in preventing atheroma. In New Zealand

white rabbits, terutroban induces regression of atherosclerotic lesions of the aorta detected by magnetic resonance imaging. The concomitant reduction in indexes of inflammation into the lesions, such as macrophages, apoptotic cells, matrix metalloproteinase-1, endothelin-1, suggests that selective inhibition of TP receptor may shift toward a more stable plaque phenotype (Chamorro 2009). Moreover, injury-induced vascular proliferation is enhanced in mice genetically deficient in the PGI_2 receptor and is reduced in mice lacking the TP receptor or treated with terutroban. Lack of both prostanoid receptors abolishes postinjury restenosis (Cheng et al. 2002).

Endogenous TP agonists are produced by the vascular endothelium, especially under pathological conditions, causing endothelium-dependent contraction and contributing to endothelial dysfunction. Therefore, TP antagonists may counteract endothelial dysfunction in diseases such as hypertension and diabetes (Feletou et al. 2010). In $apoE^{-/-}$ mice with streptozotocin-induced diabetes, terutroban reduces aortic atherosclerotic area, with improvement of endothelium-dependent relaxations to acetylcholine (Santilli et al. 2011). Thus, TP antagonism may attenuate inflammation and atherogenesis in experimental diabetes.

Administration of terutroban to coronary artery disease or to high-cardiovascular-risk patients on top of aspirin treatment improved endothelial function assessed by measuring flow-mediated dilatation (FMD) in the brachial artery (Belhassen et al. 2003; Lesault et al. 2011). The beneficial effect of terutroban on FMD was detectable after the first intake and persisted up to the end of the 2-week treatment period (Lesault et al. 2011). Thus, TP antagonists can inhibit prostanoid-mediated vasoconstriction associated with aging and/or cardiovascular risk factors related to increased oxidative stress and consequent up-regulation of COX-1 and/or induction of COX-2 (Giannarelli et al. 2010).

5.3 Ischemic Stroke: The Reasons of a Choice

The at least theoretical advantages on platelet inhibition and the actions far beyond its antithrombotic effect, including the antiproliferative and antiatherogenic properties, raised the hypothesis that TP-receptor antagonism could play a role in the clinical prevention of ischemic stroke. Preclinical findings supported this concept, indicating a greater beneficial effect of TP receptor inhibition over aspirin in a rat model of ischemic stroke (Gelosa et al. 2010). In a double-blind, parallel group study in patients with previous ischemic stroke and/or carotid stenosis, terutroban showed an antithrombotic activity superior to aspirin and similar to clopidogrel plus aspirin (Bal Dit Sollier et al. 2009). These encouraging data were the basis for undertaking the *Prevention of cerebrovascular and cardiovascular Events of ischemic origin with teRutroban in patients with a history oF ischemic strOke or tRansient ischaeMic attack* (PERFORM) study (Bousser et al. 2009).

This trial was designed to demonstrate the superiority of terutroban over aspirin in secondary prevention of cerebrovascular and cardiovascular events among ~20,000 patients with stroke. The trial (ISRCTN66157730) was recently halted on the basis of an interim analysis failing to support the superiority hypothesis, after 19,120 patients were randomly assigned, with a mean follow-up of 28·3 months (Bousser et al. 2011).

6 Great Expectations Disappointed: Did Terutroban Fail to Perform, or PERFORM Did Fail?

The PERFORM was stopped prematurely for futility after 19,120 patients were randomly assigned, with a mean follow-up of 28 months (Bousser et al. 2011). The investigators recorded no difference between terutroban and aspirin in the composite vascular primary end point, or any of the secondary or tertiary end points. However, the rate of minor bleeding was slightly increased with terutroban.

This apparent discrepancy versus the above-mentioned "great expectations" around terutroban, supported by a large body of preclinical and clinical evidence and pathophysiological rationale, draws attention and raises concerns about the interpretation of the encouraging preclinical data, as well as about the design of the clinical trial testing the superiority hypothesis of this drug versus aspirin.

6.1 Preliminary Data Revisited

The negative results of the PERFORM trial failed to come up to the expectations based on the rationale and the preliminary data supporting the superiority of terutroban over aspirin.

Both the anticipated superior antithrombotic effect and the peculiar antiatherogenic and antivasoconstrictive properties of TP antagonism need to be reconsidered in light of the clinical evidence.

Most of the data supporting a more potent antiplatelet effect of terutroban over aspirin relied upon ex vivo measurements of platelet function, such as optical aggregation to classical agonists and models of thrombus formation (Bal Dit Sollier et al. 2009; Fiessinger et al. 2010).

The apparent gap between these premises and the findings of PERFORM draws attention to the limitations of ex vivo measurements of platelet function in the characterization of platelet activation and inhibition in vivo: less than ideal intrasubject and intersubject variability, poor reproducibility on repeated measurements, variability of the TX-independent component of the different aggregation signals (Santilli et al. 2009).

Moreover, measurements of platelet function ex vivo provide an index of capacity that by no means reflects the extent of platelet activation and inhibition in vivo. Mechanism-based biochemical measurements would provide a more faithful estimation of the antiplatelet effect of aspirin.

As earlier mentioned, another war horse supporting the expected superiority of terutroban over aspirin stands in the preservation of the ability of the vasculature to synthesize the cardioprotective eicosanoid PGI_2, an important homeostatic mechanism of endothelial thromboresistance. However, the low-doses of aspirin (100 mg daily) employed vs. terutroban in the PERFORM trial have been previously shown to only marginally reduce systemic PGI_2 biosynthesis in heart failure and ischemic heart disease patients, counterbalanced by a profound reduction in TX biosynthesis (Santilli et al. 2010), consistent with the relative COX-1 selectivity achieved by a once-daily regimen of low-dose aspirin (Patrono et al. 2008) and the primary role of COX-2 in PGI_2 biosynthesis.

The antivasoconstrictive effect of terutroban, increasing blood flow through enhanced endothelium-dependent vasodilation, had the theoretical potential to affect the incidence of ischemic stroke in the PERFORM population, where traditional cardiovascular risk factors associated with endothelial dysfunction are highly prevalent. However, it has to be acknowledged that, despite several reports (Santos-Garcia et al. 2009) suggest an association, no data until now show conclusive evidence of a direct relation between endothelium-dependent contractions and the risk of ischemic stroke.

Similarly, the antiproliferative properties of terutroban, shown in a murine model of vascular injury-induced proliferation of the carotid artery, did not translate into a clinical benefit. This result might have been anticipated by the failure to prevent postcoronary angioplasty restenosis by previously developed TX-prostaglandin receptor antagonists, the CARPORT and the M-HEART (Savage et al. 1995; Serruys et al. 1991). However, none of these two studies were free of limitations, the first being an uncontrolled experience relying on a single measurement of an angiographic end point, the second including no measure of the degree of synthesis inhibition achieved by aspirin or of TP blockade by either antagonist. Moreover, the M-HEART based the superiority of terutroban over aspirin on the ability of terutroban to preserve the antiproliferative effect of PGI_2 biosynthesis during angiography, which was short lived and suppressed by the concurrent aspirin treatment in all patients undergoing PCI (Praticó et al. 2000).

The antiatherogenic properties of TP antagonists were considered as a relevant plus, stimulating the preferential recruitment of patients with an atherothrombotic cerebral ischemic event. However, even in this subgroup, no benefit was recorded for terutroban compared with aspirin in the PERFORM, possibly because their atheromatous lesions were already well advanced. This assumption is supported by a study performed in a murine model of atherogenesis, showing that TP antagonism inhibits initiation and early development of atherosclerotic lesions in mice, but failed to induce regression of established atherosclerotic disease (Egan et al. 2005). Thus, the clinical use of TP antagonists would be expected to be useful in the earlier stages of disease, rather than in reversing accumulated plaque burden in patients with diffuse, established atherosclerosis.

6.2 The PERFORM Design Revisited

A few lessons might be drawn by the comparison of PERFORM findings with similar trials of other antiplatelets vs. aspirin. PERFORM is the second largest secondary prevention trial of an antiplatelet drug undertaken so far in patients with cerebral ischemic events. The largest study, PROFESS (Sacco et al. 2008), compared aspirin plus extended-release dipyridamole and clopidogrel in 20,232 patients followed up for a mean of 30 months. This trial showed similar rates of recurrent stroke with aspirin plus extended-release dipyridamole and with clopidogrel. Failure to achieve the superiority goal in both trials raises concerns about the clinical setting in which selective TP receptor blockade might confer an advantage over low-dose aspirin. Ischemic stroke might be a difficult setting as compared to MI, but no trial so far is available to confirm or reject this speculative hypothesis.

Furthermore, the comparative analysis versus previously performed trials in the same setting unravels the likelihood that the event rate observed in the PERFORM might be someway lower than expected, thus affecting the statistical power of the study. Although recent trials (ESPRIT and CSPS 2) have shown similar rates of strokes with aspirin to those seen in PERFORM (Halkes et al. 2006; Shinohara et al. 2010), the stroke rate with aspirin in PERFORM should have been much higher because of the enrolment of several patients during the period of highest risk for recurrence (less than 3 months since the qualifying event) and because 52% of the qualifying strokes were due to the stroke subtype with the poorer prognosis, atherothrombosis (compared with about 30% in ESPRIT and CSPS 2). Even in the PROFESS trial, which, similar to the PERFORM, enrolled patients who had experienced an ischemic stroke for less than 90 days, the event rates were remarkably close to PERFORM, but the proportion of atherothrombotic infarcts was significantly higher (67% vs. 28%) in PERFORM.

The choice of the lag time after the qualifying event, similar to the PROFESS trial, might also have affected the effectiveness of the drug: in fact, patients could be enrolled after the index event in PERFORM much sooner than in other recent antiplatelet trials. While aspirin has proven benefits when given early after a stroke (Chen et al. 2000), the efficacy of this strategy for other antiplatelets has not been definitively proven.

The choice of the dose (30 mg once daily) has been an additional issue advocated to explain the drug failure to exert superior effects on vascular events than aspirin. However, the slight excess in minor bleeding with this dose suggests that, even if higher doses may be more appropriate on the efficacy side, this benefit could be offset by more hemorrhagic episodes, which seem proportional to the number of TX-prostaglandin receptors bound by the drug.

The duration of follow-up appears as another critical and still unanswered issue: given that only 15% of patients were followed up to or beyond 3 years, a difference in treatment effect might have emerged later, since additional potential longer term effects of terutroban cannot be excluded.

As previously shown for aspirin (Baigent et al. 2009), improving background risk-factor control could have blunted the ability of terutroban to outperform aspirin. Nowadays, many patients with a history of occlusive vascular events would have their risks of recurrence reduced substantially by statins, other modern drugs, and vascular procedures. If so, and if the other interventions approximately halve the recurrence risk, then the absolute benefit of adding an antiplatelet to these other methods might be only about half as great as that of giving an antiplatelet alone.

Finally, the observation that patients with a history of ischemic stroke before the qualifying event had a lower event rate with terutroban than with aspirin is worth of further attention. Although this finding could be attributable to chance, it is plausible that most of these patients would have been receiving aspirin before their PERFORM qualifying event, thus the switch to a different antiplatelet drug appears as a more effective strategy than continuation of aspirin despite having experienced an event while on aspirin. Lower than expected response to aspirin which translates into clinical failure might be an expected and relevant phenomenon in particular subgroups of patients, such as those with diabetes mellitus (representing 27% and 28% of each arm, respectively). Trials that randomly assign patients with a breakthrough event while on aspirin to a newer antiplatelet drug or to a different dosing regimen, rather than to the original aspirin dose, could provide insights into this issue. Perhaps terutroban could be tested for this specific issue or in clinical settings where a benefit is anticipated, such as diabetes mellitus. In the PERFORM, only about one out of five of the patient population had diabetes, thus potentially affecting the statistical power of the study to test the superiority hypothesis in diabetes.

7 Future Perspectives

As earlier mentioned, failure of the PERFORM does not preclude the possibility that TP receptor antagonist may be an effective tool in the prevention of vascular events in other clinical settings, such as diabetes, where the pathophysiological premises for a beneficial role look more sound. This might be worth of ad hoc trials, although the PERFORM findings are likely to discourage any other drug company from pursuing this drug target.

Moving from the cardiovascular setting, an increasing body of evidence provides the rationale for a role of TXS and TP receptor signaling in carcinogenesis, which may arise as a “rescue” setting for the clinical development of these drugs.

TXS signaling has been implicated in the development and progression of cancer, by acting on a range of tumor cell survival pathways, such as tumor cell proliferation, induction of apoptosis, invasion and metastasis, and tumor cell angiogenesis (Cathcart et al. 2010).

Increased expression of TXS and TP-β isoform has been found in the tissues of patients with bladder cancer. TP-β receptor overexpression in patients with bladder

cancer is associated with a poorer prognosis (Moussa et al. 2008). Studies in cell lines and mice have indicated a potential significant role of the TX signaling pathway in the pathogenesis of human bladder cancer. Stimulation of TP receptors is associated with a mitogenic response and in phosphorylation of several kinases. TP receptor antagonists augment in vitro and in vivo responses to cisplatin, by reducing cell proliferation in vitro, increasing the time of tumor onset, and reducing the rate of tumor growth in vivo (Moussa et al. 2008). These studies raise the possibility that the TP-β receptor could serve as a novel therapeutic target in bladder cancer and its presence and/or overexpression could be used as a predictor of prognosis and dictate therapy. Recently, increased tissue levels of the TP-β receptor in patients with bladder cancer have been found to be mirrored by increased urinary levels of TXB_2, the major metabolite of TXA_2, suggesting that patients with bladder cancer may be followed for progression or remission of their disease by quantitation of these substances in their urine (Moussa et al. 2011).

TXS is also overexpressed in nonsmall cell lung cancer (NSCLC), particularly in the adenocarcinoma subtype. Selective TXS inhibition prevents proliferation and induces apoptosis (Cathcart et al. 2011).

Clearly, further studies are needed to delineate the role of TXS and TP-β receptors in cancer and to address the challenge of their pharmacological inhibition through a clinical development program.

8 Conclusions and Implications for Clinical Usefulness of TP Antagonists

Several clinical conditions are characterized by increased incidence of cardiovascular events and enhanced TX-dependent platelet activation.

Aspirin is thought to be the best choice in these settings. However, the optimum regimen to suppress TX formation remains undefined. In fact, enhanced TX generation may be explained by mechanisms relatively insensitive to aspirin.

Extraplatelet, nucleate sources of TXA_2 biosynthesis, possibly triggered by inflammatory stimuli, and F2-isoprostane formation, reflecting ongoing in vivo oxidative stress, can activate platelets via the platelet TP receptor thus escaping inhibition of aspirin (Davì et al. 1990).

Thus, the relevance of the TP receptor in the pathogenesis of vascular diseases, particularly in diabetes, may be due to the fact that not only TXA_2, but other eicosanoids including HETEs and isoprostanes are produced to such an extent as to activate the TP receptor. Aspirin has no effect on isoprostanes which are formed nonenzymatically from AA, and aspirin can actually increase HETE production by COX (Meade et al. 1993).

In clinical settings characterized by enhanced platelet generation, younger reticulated platelets are increased, and platelet COX-2 expression is up-regulated and a consistent TX production may be driven by this enzymatic pathway, relatively insensitive to aspirin (Guthikonda et al. 2007; Santilli et al. 2009).

An antithrombotic intervention blocking TP may be required, as a common downstream pathway for both platelet and extraplatelet TXA_2 as well as for isoprostanes. Aspirin does not inhibit isoprostane formation. Moreover, intraplatelet or extraplatelet TX generation may be only partly inhibited by aspirin under certain pathological conditions, at least at the usual low doses given for cardiovascular protection.

Moreover, a TP antagonist has actions far beyond its antithrombotic effect exerted on platelets and can be attributed to direct effects on endothelial and smooth muscle cells within the blood vessel wall. These include effects on vascular adhesion molecules, NO synthase expression and function, oxidant production, and accumulation of extracellular matrix and advanced glycation end-products.

Thus, TP antagonists may represent an ideal tool to improve our knowledge on the pathophysiology of cardiovascular diseases and to improve our pharmacological "weapons" to counteract them in clinical settings, such as diabetes mellitus, characterized by persistent enhanced TXA_2-dependent platelet activation.

Knowledge Gaps

- The prevention of vascular events by TP receptor antagonists in clinical settings, such as diabetes, where the pathophysiological premises for a beneficial role look more sound needs to be defined.
- The hypothesis that TP-β receptor could serve as a novel therapeutic target in bladder cancer and its presence and/or overexpression could be used as a predictor of prognosis and dictate therapy needs to be tested.
- More in general, further studies are needed to delineate the role of TXS and TP-β receptors in cancer and to address the challenge of their pharmacological inhibition through a clinical development program.

Key Messages

- Thromboxane synthase (TXS) inhibitors and thromboxane receptor (TP) antagonists have the potential to prove more effective than aspirin due to their different mechanism of action along the pathway of TXA_2. TXS inhibitors prevent the conversion of PGH_2 to TXA_2, reducing TXA_2 synthesis mainly in platelets, whereas TP antagonists block the downstream consequences of TP activation.
- Targeting TP receptor, a common downstream pathway for both platelet and extraplatelet TXA_2 as well as for endoperoxides and isoprostanes, may be a useful antiatherosclerotic and a more powerful antithrombotic intervention in clinical settings, such as diabetes mellitus, characterized by persistently enhanced TX-dependent platelet activation through isoprostane formation and low-grade inflammation, leading to extraplatelet sources of TXA_2.

(continued)

- Despite great expectations on this drug supported by a large body of preclinical and clinical evidence and pathophysiological rationale, the PERFORM trial failed to demonstrate the superiority of terutroban over aspirin in secondary prevention of cerebrovascular and cardiovascular events among ~20,000 patients with stroke. However, the clinical setting and the design of the study in which the drug has been challenged, as well as a sometimes uncritical translation of preclinical data into a rationale for a clinical trial, may explain, at least in part, this unexpected finding.
- Drugs with dual action, such as dual TXS inhibitors/TP antagonist and dual COXIB/TP antagonists are currently in clinical development. Besides the theoretical rationale for their benefit, ongoing clinical studies are challenging their potential.

References

Ali S, Davis MG, Becker MW, Dorn GW 2nd (1993) Thromboxane A2 stimulates vascular smooth muscle hypertrophy by up-regulating the synthesis and release of endogenous basic fibroblast growth factor. J Biol Chem 268:17397–17403

Ashton AW, Ware GM, Kaul DK, Ware JA (2003) Inhibition of tumor necrosis factor alpha-mediated NFkappaB activation and leukocyte adhesion, with enhanced endothelial apoptosis, by G protein-linked receptor (TP) ligands. J Biol Chem 278:11858–11866

Baigent C, Blackwell L, Collins R, Emberson J, Godwin J, Peto R, Buring J, Hennekens C, Kearney P, Meade T, Patrono C, Roncaglioni MC, Zanchetti A (2009) Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet 373:1849–1860

Bal Dit Sollier C, Crassard I, Simoneau G, Bergmann JF, Bousser MG, Drouet L (2009) Effect of the thromboxane prostaglandin receptor antagonist terutroban on arterial thrombogenesis after repeated administration in patients treated for the prevention of ischemic stroke. Cerebrovasc Dis 28:505–513

Basili S, Raparelli V, Vestri A, Di Tanna GL, Violi F (2010) Comparison of efficacy of antiplatelet treatments for patients with claudication. A meta-analysis. Thromb Haemost 103:766–773

Belhassen L, Pelle G, Dubois-Rande JL, Adnot S (2003) Improved endothelial function by the thromboxane A2 receptor antagonist S 18886 in patients with coronary artery disease treated with aspirin. J Am Coll Cardiol 41:1198–1204

Bousser MG, Amarenco P, Chamorro A, Fisher M, Ford I, Fox K, Hennerici MG, Mattle HP, Rothwell PM (2009) Rationale and design of a randomized, double-blind, parallel-group study of terutroban 30 mg/day versus aspirin 100 mg/day in stroke patients: the prevention of cerebrovascular and cardiovascular events of ischemic origin with terutroban in patients with a history of ischemic stroke or transient ischemic attack (PERFORM) study. Cerebrovasc Dis 27:509–518

Bousser MG, Amarenco P, Chamorro A, Fisher M, Ford I, Fox KM, Hennerici MG, Mattle HP, Rothwell PM, de Cordoue A, Fratacci MD (2011) Terutroban versus aspirin in patients with cerebral ischaemic events (PERFORM): a randomised, double-blind, parallel-group trial. Lancet 377:2013–2022

Cathcart MC, Reynolds JV, O'Byrne KJ, Pidgeon GP (2010) The role of prostacyclin synthase and thromboxane synthase signaling in the development and progression of cancer. Biochim Biophys Acta 1805:153–166

Cathcart MC, Gately K, Cummins R, Kay E, O'Byrne KJ, Pidgeon GP (2011) Examination of thromboxane synthase as a prognostic factor and therapeutic target in non-small cell lung cancer. Mol Cancer 10:25

Cayatte AJ, Du Y, Oliver-Krasinski J, Lavielle G, Verbeuren TJ, Cohen RA (2000) The thromboxane receptor antagonist S18886 but not aspirin inhibits atherogenesis in apo E-deficient mice: evidence that eicosanoids other than thromboxane contribute to atherosclerosis. Arterioscler Thromb Vasc Biol 20:1724–1728

Chamorro A (2009) TP receptor antagonism: a new concept in atherothrombosis and stroke prevention. Cerebrovasc Dis 27(Suppl 3):20–27

Chen ZM, Sandercock P, Pan HC, Counsell C, Collins R, Liu LS, Xie JX, Warlow C, Peto R (2000) Indications for early aspirin use in acute ischemic stroke: a combined analysis of 40 000 randomized patients from the chinese acute stroke trial and the international stroke trial. On behalf of the CAST and IST collaborative groups. Stroke 31:1240–1249

Cheng Y, Austin SC, Rocca B, Koller BH, Coffman TM, Grosser T, Lawson JA, FitzGerald GA (2002) Role of prostacyclin in the cardiovascular response to thromboxane A2. Science 296:539–541

Davì G, Falco A (2005) Oxidant stress, inflammation and atherogenesis. Lupus 14:760–764

Davì G, Patrono C (2007) Platelet activation and atherothrombosis. N Engl J Med 357:2482–2494

Davì G, Catalano I, Averna M, Notarbartolo A, Strano A, Ciabattoni G, Patrono C (1990) Thromboxane biosynthesis and platelet function in type II diabetes mellitus. N Engl J Med 322:1769–1774

Davì G, Ciabattoni G, Consoli A, Mezzetti A, Falco A, Santarone S, Pennese E, Vitacolonna E, Bucciarelli T, Costantini F, Capani F, Patrono C (1999) In vivo formation of 8-iso-prostaglandin f2alpha and platelet activation in diabetes mellitus: effects of improved metabolic control and vitamin E supplementation. Circulation 99:224–229

Dogne JM, de Leval X, Hanson J, Frederich M, Lambermont B, Ghuysen A, Casini A, Masereel B, Ruan KH, Pirotte B, Kolh P (2004) New developments on thromboxane and prostacyclin modulators part I: thromboxane modulators. Curr Med Chem 11:1223–1241

Dogne JM, Hanson J, Pratico D (2005) Thromboxane, prostacyclin and isoprostanes: therapeutic targets in atherogenesis. Trends Pharmacol Sci 26:639–644

Egan KM, Wang M, Fries S, Lucitt MB, Zukas AM, Pure E, Lawson JA, FitzGerald GA (2005) Cyclooxygenases, thromboxane, and atherosclerosis: plaque destabilization by cyclooxygenase-2 inhibition combined with thromboxane receptor antagonism. Circulation 111:334–342

Eikelboom JW, Hirsh J, Weitz JI, Johnston M, Yi Q, Yusuf S (2002) Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. Circulation 105:1650–1655

Feletou M, Verbeuren TJ, Vanhoutte PM (2009) Endothelium-dependent contractions in SHR: a tale of prostanoid TP and IP receptors. Br J Pharmacol 156:563–574

Feletou M, Cohen RA, Vanhoutte PM, Verbeuren TJ (2010) TP receptors and oxidative stress hand in hand from endothelial dysfunction to atherosclerosis. Adv Pharmacol 60:85–106

Ferroni P, Basili S, Falco A, Davì G (2004) Platelet activation in type 2 diabetes mellitus. J Thromb Haemost 2:1282–1291

Fiessinger JN, Bounameaux H, Cairols MA, Clement DL, Coccheri S, Fletcher JP, Hoffmann U, Turpie AG (2010) Thromboxane Antagonism with terutroban in Peripheral Arterial Disease: the TAIPAD study. J Thromb Haemost 8:2369–2376

FitzGerald GA, Reilly IA, Pedersen AK (1985) The biochemical pharmacology of thromboxane synthase inhibition in man. Circulation 72:1194–1201

Gaussem P, Reny JL, Thalamas C, Chatelain N, Kroumova M, Jude B, Boneu B, Fiessinger JN (2005) The specific thromboxane receptor antagonist S18886: pharmacokinetic and pharmacodynamic studies. J Thromb Haemost 3:1437–1445

Gelosa P, Ballerio R, Banfi C, Nobili E, Gianella A, Pignieri A, Brioschi M, Guerrini U, Castiglioni L, Blanc-Guillemaud V, Lerond L, Tremoli E, Sironi L (2010) Terutroban, a

thromboxane/prostaglandin endoperoxide receptor antagonist, increases survival in stroke-prone rats by preventing systemic inflammation and endothelial dysfunction: comparison with aspirin and rosuvastatin. J Pharmacol Exp Ther 334:199–205

Giannarelli C, Zafar MU, Badimon JJ (2010) Prostanoid and TP-receptors in atherothrombosis: is there a role for their antagonism? Thromb Haemost 104:949–954

Guthikonda S, Lev EI, Patel R, DeLao T, Bergeron AL, Dong JF, Kleiman NS (2007) Reticulated platelets and uninhibited COX-1 and COX-2 decrease the antiplatelet effects of aspirin. J Thromb Haemost 5:490–496

Halkes PH, van Gijn J, Kappelle LJ, Koudstaal PJ, Algra A (2006) Aspirin plus dipyridamole versus aspirin alone after cerebral ischaemia of arterial origin (ESPRIT): randomised controlled trial. Lancet 367:1665–1673

Ishizuka T, Suzuki K, Kawakami M, Hidaka T, Matsuki Y, Nakamura H (1996) Thromboxane A2 receptor blockade suppresses intercellular adhesion molecule-1 expression by stimulated vascular endothelial cells. Eur J Pharmacol 312:367–377

Ishizuka T, Kawakami M, Hidaka T, Matsuki Y, Takamizawa M, Suzuki K, Kurita A, Nakamura H (1998) Stimulation with thromboxane A2 (TXA2) receptor agonist enhances ICAM-1, VCAM-1 or ELAM-1 expression by human vascular endothelial cells. Clin Exp Immunol 112:464–470

Kiff PS, Bergman G, Atkinson L, Jewitt DE, Westwick J, Kakkar VV (1983) Haemodynamic and metabolic effects of dazoxiben at rest and during atrial pacing. Br J Clin Pharmacol 15:73S–77S

Lesault PF, Boyer L, Pelle G, Covali-Noroc A, Rideau D, Akakpo S, Teiger E, Dubois-Rande JL, Adnot S (2011) Daily administration of the TP receptor antagonist terutroban improved endothelial function in high-cardiovascular-risk patients with atherosclerosis. Br J Clin Pharmacol 71:844–851

Mayeux PR, Morton HE, Gillard J, Lord A, Morinelli TA, Boehm A, Mais DE, Halushka PV (1988) The affinities of prostaglandin H2 and thromboxane A2 for their receptor are similar in washed human platelets. Biochem Biophys Res Commun 157:733–739

Meade EA, Smith WL, DeWitt DL (1993) Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. J Biol Chem 268:6610–6614

Meadows TA, Bhatt DL (2007) Clinical aspects of platelet inhibitors and thrombus formation. Circ Res 100:1261–1275

Mehta P, Mehta J, Lawson D, Krop I, Letts LG (1986) Leukotrienes potentiate the effects of epinephrine and thrombin on human platelet aggregation. Thromb Res 41:731–738

Meja KK, Barnes PJ, Giembycz MA (1997) Characterization of the prostanoid receptor(s) on human blood monocytes at which prostaglandin E2 inhibits lipopolysaccharide-induced tumour necrosis factor-alpha generation. Br J Pharmacol 122:149–157

Miggin SM, Kinsella BT (1998) Expression and tissue distribution of the mRNAs encoding the human thromboxane A2 receptor (TP) alpha and beta isoforms. Biochim Biophys Acta 1425:543–559

Moussa O, Ashton AW, Fraig M, Garrett-Mayer E, Ghoneim MA, Halushka PV, Watson DK (2008) Novel role of thromboxane receptors beta isoform in bladder cancer pathogenesis. Cancer Res 68:4097–4104

Moussa O, Ciupek A, Watson DK, Halushka PV (2011) Urinary thromboxane B(2) and thromboxane receptors in bladder cancer: opportunity for detection and monitoring. Prostaglandins Other Lipid Mediat 96(1–4):41–44

Nakahata N (2008) Thromboxane A2: physiology/pathophysiology, cellular signal transduction and pharmacology. Pharmacol Ther 118:18–35

Neri Serneri GG, Coccheri S, Marubini E, Violi F (2004) Picotamide, a combined inhibitor of thromboxane A2 synthase and receptor, reduces 2-year mortality in diabetics with peripheral arterial disease: the DAVID study. Eur Heart J 25:1845–1852

Patrono C (1990) Biosynthesis and pharmacological modulation of thromboxane in humans. Circulation 81:I12–I15, discussion I22–I23

Patrono C, Falco A, Davì G (2005a) Isoprostane formation and inhibition in atherothrombosis. Curr Opin Pharmacol 5:198–203

Patrono C, Garcia Rodriguez LA, Landolfi R, Baigent C (2005b) Low-dose aspirin for the prevention of atherothrombosis. N Engl J Med 353:2373–2383

Patrono C, Baigent C, Hirsh J, Roth G (2008) Antiplatelet drugs: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest 133:199S–233S

Patscheke H (1990) Thromboxane A2/prostaglandin H2 receptor antagonists. A new therapeutic principle. Stroke 21:IV139–IV142

Praticó D, Cheng Y, FitzGerald GA (2000) TP or not TP: primary mediators in a close runoff? Arterioscler Thromb Vasc Biol 20:1695–1698

Rovati GE, Sala A, Capra V, Dahlen SE, Folco G (2010) Dual COXIB/TP antagonists: a possible new twist in NSAID pharmacology and cardiovascular risk. Trends Pharmacol Sci 31:102–107

Sacco RL, Diener HC, Yusuf S, Cotton D, Ounpuu S, Lawton WA, Palesch Y, Martin RH, Albers GW, Bath P, Bornstein N, Chan BP, Chen ST, Cunha L, Dahlof B, De Keyser J, Donnan GA, Estol C, Gorelick P, Gu V, Hermansson K, Hilbrich L, Kaste M, Lu C, Machnig T, Pais P, Roberts R, Skvortsova V, Teal P, Toni D, Vandermaelen C, Voigt T, Weber M, Yoon BW (2008) Aspirin and extended-release dipyridamole versus clopidogrel for recurrent stroke. N Engl J Med 359:1238–1251

Santilli F, Rocca B, De Cristofaro R, Lattanzio S, Pietrangelo L, Habib A, Pettinella C, Recchiuti A, Ferrante E, Ciabattoni G, Davì G, Patrono C (2009) Platelet cyclooxygenase inhibition by low-dose aspirin is not reflected consistently by platelet function assays: implications for aspirin "resistance". J Am Coll Cardiol 53:667–677

Santilli F, Davì G, Basili S, Lattanzio S, Cavoni A, Guizzardi G, De Feudis L, Traisci G, Pettinella C, Paloscia L, Minuz P, Meneguzzi A, Ciabattoni G, Patrono C (2010) Thromboxane and prostacyclin biosynthesis in heart failure of ischemic origin: effects of disease severity and aspirin treatment. J Thromb Haemost 8:914–922

Santilli F, Mucci L, Davì G (2011) TP receptor activation and inhibition in atherothrombosis: the paradigm of diabetes mellitus. Intern Emerg Med 6:203–212

Santos-Garcia D, Blanco M, Serena J, Arias S, Millan M, Rodriguez-Yanez M, Leira R, Davalos A, Castillo J (2009) Brachial arterial flow mediated dilation in acute ischemic stroke. Eur J Neurol 16:684–690

Savage MP, Goldberg S, Bove AA, Deutsch E, Vetrovec G, Macdonald RG, Bass T, Margolis JR, Whitworth HB, Taussig A (1995) Effect of thromboxane A2 blockade on clinical outcome and restenosis after successful coronary angioplasty. Multi-Hospital Eastern Atlantic Restenosis Trial (M-HEART II). Circulation 92:3194–3200

Schramm TK, Gislason GH, Kober L, Rasmussen S, Rasmussen JN, Abildstrom SZ, Hansen ML, Folke F, Buch P, Madsen M, Vaag A, Torp-Pedersen C (2008) Diabetes patients requiring glucose-lowering therapy and nondiabetics with a prior myocardial infarction carry the same cardiovascular risk: a population study of 3.3 million people. Circulation 117:1945–1954

Selg E, Buccellati C, Andersson M, Rovati GE, Ezinga M, Sala A, Larsson AK, Ambrosio M, Lastbom L, Capra V, Dahlen B, Ryrfeldt A, Folco GC, Dahlen SE (2007) Antagonism of thromboxane receptors by diclofenac and lumiracoxib. Br J Pharmacol 152:1185–1195

Serruys PW, Rutsch W, Heyndrickx GR, Danchin N, Mast EG, Wijns W, Rensing BJ, Vos J, Stibbe J (1991) Prevention of restenosis after percutaneous transluminal coronary angioplasty with thromboxane A2-receptor blockade. A randomized, double-blind, placebo-controlled trial. Coronary Artery Restenosis Prevention on Repeated Thromboxane-Antagonism Study (CARPORT). Circulation 84:1568–1580

Shikano M, Ito T, Ogawa K, Satake T (1987) Effects of a selective thromboxane synthetase inhibitor (OKY-046) in patients with coronary artery disease during exercise. Jpn Heart J 28:663–674

Shinohara Y, Katayama Y, Uchiyama S, Yamaguchi T, Handa S, Matsuoka K, Ohashi Y, Tanahashi N, Yamamoto H, Genka C, Kitagawa Y, Kusuoka H, Nishimaru K, Tsushima M, Koretsune Y, Sawada T, Hamada C (2010) Cilostazol for prevention of secondary stroke (CSPS 2): an aspirin-controlled, double-blind, randomised non-inferiority trial. Lancet Neurol 9:959–968

Swinnen M, Vanhoutte D, Van Almen GC, Hamdani N, Schellings MW, D'Hooge J, Van der Velden J, Weaver MS, Sage EH, Bornstein P, Verheyen FK, VandenDriessche T, Chuah MK, Westermann D, Paulus WJ, Van de Werf F, Schroen B, Carmeliet P, Pinto YM, Heymans S (2009) Absence of thrombospondin-2 causes age-related dilated cardiomyopathy. Circulation 120:1585–1597

Thaulow E, Dale J, Myhre E (1984) Effects of a selective thromboxane synthetase inhibitor, dazoxiben, and of acetylsalicylic acid on myocardial ischemia in patients with coronary artery disease. Am J Cardiol 53:1255–1258

The Ridogrel Versus Aspirin Patency Trial (1994) Randomized trial of ridogrel, a combined thromboxane A2 synthase inhibitor and thromboxane A2/prostaglandin endoperoxide receptor antagonist, versus aspirin as adjunct to thrombolysis in patients with acute myocardial infarction. Circulation 89:588–595

Toth P, Rozsa B, Springo Z, Doczi T, Koller A (2011) Isolated human and rat cerebral arteries constrict to increases in flow: role of 20-HETE and TP receptors. J Cereb Blood Flow Metab 31:2096–2105

Tytgat GN, Van Nueten L, Van De Velde I, Joslyn A, Hanauer SB (2002) Efficacy and safety of oral ridogrel in the treatment of ulcerative colitis: two multicentre, randomized, double-blind studies. Aliment Pharmacol Ther 16:87–99

Uehara Y, Ishimitsu T, Kimura K, Ishii M, Ikeda T, Sugimoto T (1988) Regulatory effects of eicosanoids on thymidine uptake by vascular smooth muscle cells of rats. Prostaglandins 36:847–857

Uyama O, Nagatsuka K, Nakabayashi S, Isaka Y, Yoneda S, Kimura K, Abe H (1985) The effect of a thromboxane synthetase inhibitor, OKY-046, on urinary excretion of immunoreactive thromboxane B2 and 6-keto-prostaglandin F1 alpha in patients with ischemic cerebrovascular disease. Stroke 16:241–244

Vandeplassche G, Hermans C, Somers Y, Van de Werf F, de Clerck F (1993) Combined thromboxane A2 synthase inhibition and prostaglandin endoperoxide receptor antagonism limits myocardial infarct size after mechanical coronary occlusion and reperfusion at doses enhancing coronary thrombolysis by streptokinase. J Am Coll Cardiol 21:1269–1279

Violi F, Hiatt W (2007) A critical review of antiplatelet treatment in peripheral arterial disease. Intern Emerg Med 2:84–87

Watala C, Boncler M, Gresner P (2005) Blood platelet abnormalities and pharmacological modulation of platelet reactivity in patients with diabetes mellitus. Pharmacol Rep 57 (Suppl):42–58

Yui Y, Hattori R, Takatsu Y, Nakajima H, Wakabayashi A, Kawai C, Kayama N, Hiraku S, Inagawa T, Tsubojima M et al (1984) Intravenous infusion of a selective inhibitor of thromboxane A2 synthetase in man: influence on thromboxane B2 and 6-keto-prostaglandin F1 alpha levels and platelet aggregation. Circulation 70:599–605

Zuccollo A, Shi C, Mastroianni R, Maitland-Toolan KA, Weisbrod RM, Zang M, Xu S, Jiang B, Oliver-Krasinski JM, Cayatte AJ, Corda S, Lavielle G, Verbeuren TJ, Cohen RA (2005) The thromboxane A2 receptor antagonist S18886 prevents enhanced atherogenesis caused by diabetes mellitus. Circulation 112:3001–3008