Antiplatelet therapy: thrombin receptor antagonists

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Activated platelets stimulate thrombus formation in response to rupture of an atherosclerotic plaque or endothelial cell erosion, promoting atherothrombotic disease. Multiple pathways contribute to platelet activation. Aspirin, an irreversible inhibitor of thromboxane A₂ synthesis, in combination with clopidogrel, an inhibitor of P2Y₁₂ adenosine diphosphate platelet receptors, represent the current standard-of-care of antiplatelet therapy for patients with acute coronary syndrome and for those undergoing percutaneous coronary intervention. Although these agents have demonstrated significant clinical benefit, the increased risk of bleeding and the recurrence of thrombotic events represent substantial limitations. Thrombin is one of the most important platelet activators. The inhibition of protease-activated receptor 1 showed a good safety profile in preclinical studies. In fact, phase II studies with vorapaxar (SCH530348) and atopaxar (E5555) showed no increase of bleeding events in addition to the current standard-of-care of antiplatelet therapy. Although the results of phase III trials for both drugs are awaited, this family is a promising new addition to the current clinical practice for patients with atherothrombotic disease, not only as an alternative, but also as additional therapy.

Introduction

The use of an inhibitor of platelet activation via synthesis of thromboxane A₂ (TXA₂; aspirin) and a P2Y₁₂ adenosine diphosphate (ADP) receptor inhibitor (clopidogrel) represents the standard-of-care for the treatment of recurrent thrombotic complications of patients with manifestations of atherosclerotic disease [1]. A large number of studies have shown the benefit of aspirin in the setting of different acute clinical scenarios, such as acute coronary syndrome (ACS) [2, 3] and percutaneous revascularization [4]. Furthermore, several studies have also shown the benefit of aspirin in the maintenance phase of these diseases as secondary prevention [5] and as primary prevention in patients with cardiovascular risk factors [2]. In contrast, the clinical efficacy of clopidogrel has been shown both as a single antiplatelet therapy in high-risk patients with various manifestations of atherosclerotic disease [6] and in combination with aspirin in patients across the ACS spectrum (unstable angina, non-ST elevation myocardial infarction and ST elevation myocardial infarction) or in those undergoing percutaneous coronary intervention (PCI) [7–12]. However, recurrent thrombotic events may continue to occur despite use of standard dual antiplatelet treatment regimens. Several reasons may explain this phenomenon, the most

important of which appear to be the following: (i) there are multiple pathways contributing to platelet activation and aggregation processes, and aspirin and P2Y₁₂ ADP receptor antagonists do not inhibit pathways other than those stimulated by TXA₂ and ADP, leaving other pivotal pathways (e.g. thrombin-mediated pathways) uninhibited; and (ii) a considerable number of patients may have inadequate responsiveness to aspirin and clopidogrel therapy; this phenomenon has been shown in several studies to be associated with an increased risk of recurrent ischaemic events [13–15]. Description of the latter phenomenon goes beyond the scope of this review. The present review article provides an overview of the rationale for the use of thrombin receptor-inhibiting strategies as well as the currently available results from clinical investigations and future directions in the field. [16].

Role of platelets in thrombosis and haemostasis: basic principles and implications for thrombin-mediated signalling

The important role of platelets in normal haemostatic processes and in atherothrombotic disease are well known (Figure 1) [17]. Following plaque rupture/erosion, which

Figure 1

Platelet adhesion and aggregation on the extracellular matrix. The interaction between glycoprotein (GP) Ib and von Willebrand factor (vWF) mediates platelet tethering, and this enables subsequent interaction between GP VI and collagen. This triggers the shift of integrins to a high-affinity state and the release of adenosine diphosphate (ADP) and thromboxane A₂ (TXA₂), which bind to the P2Y₁₂ and TP receptors, respectively. Tissue factor (TF) locally triggers thrombin formation, which also contributes to platelet activation via binding to the platelet protease-activated receptor 1 (PAR-1). Adapted from reference [29]. vWF (blue Y-shape); GP Ib-V-IX (red Y-shape); GP VI (green Y-shape); Fibrinogen (red Y-shape); Resting GP IIb/IIIa (purple Y-shape); Activated GP IIb/IIIa (pink Y-shape); Resting α₂β₁ (blue Y-shape); Activated α₂β₁ (pink Y-shape); P2Y₁₂ (green Y-shape); TP receptor (red Y-shape); PAR-1 (blue Y-shape). TP, thromboxane receptor

may either occur spontaneously, e.g. in patients with an acute coronary event, or be iatrogenically induced, e.g. in patients undergoing percutaneous coronary intervention, platelet adhesion to the extracellular matrix occurs, which is mediated primarily by collagen receptors. After the initial adhesion of platelets to the extracellular matrix, the repair process requires a rapid response to autocrine and paracrine mediators, including ADP, thrombin, adrenaline and TXA₂. These mediators amplify and sustain the initial platelet response and they recruit circulating platelets from the bloodstream to form a growing haemostatic plug [18]. Briefly, there are three main platelet membrane receptors to drive platelet activation (and maintain the response): TXA₂ receptors, ADP receptors and thrombin receptors or protease-activated receptors (PARs) [18]. These are G protein-coupled receptors expressed by platelets and they mediate paracrine and autocrine platelet activation. Platelet activation by these factors leads to a change in platelet shape [19–21], expression of proinflammatory molecules, such as P-selectin [19], soluble CD40 ligand (sCD40L) [22] and other unidentified proteins [23], expression of platelet procoagulant activity [24], potentiation of aggregation by other prothrombotic factors, such as collagen [25], and, finally and most importantly, the conversion of the platelet receptor glycoprotein (GP) IIb/IIIa into an active form, which allows platelet-to-platelet adhesion, thus mediating the final aggregation process [18]. As thrombin is described as the most potent platelet activator [26, 27], inhibition of PARs provides a

rational approach to define antiplatelet agents that more effectively inhibit platelet activation. The limitations of current therapies underscore the need for identifying other antiplatelet strategies to provide more comprehensive platelet inhibition.

Thrombin-induced platelet activation: role of PARs

The PARs have a well-established role in vascular biology, haemostasis and atherothrombosis as the receptors for thrombin. The serine protease thrombin is not only a key enzyme of the coagulation cascade but is also the strongest platelet agonist and is therefore an important contributor to atherothrombotic processes [28, 29]. The PARs are G protein-coupled receptors that mediate the cellular effects of different proteases, including thrombin. The proteolytic enzyme cleaves the extracellular loop of the G protein-coupled receptor, and the newly unmasked N-terminus acts as a tethered ligand that binds to the proximally located transmembrane loop of these receptors [30].

The mechanism of PAR activation and signalling is complex. Thrombin is an enzyme, and thus it can activate more than one molecule of the receptor. Another peculiarity is that the proteolytic cleavage of the receptor by thrombin is irreversible, and finally, the proteolytic cleav-

Figure 2

Pathways of platelet protease-activated receptor 1 (PAR-1) activation. Activated PAR-1 can signal through Gα12/13, Gαq and Gβγ families. The α-subunits of G12 and 13 bind Rho GEFs and induce Rho-mediated cytoskeletal responses leading to changes in platelet shape. The Gαq binds phospholipase Cβ to generate IP3, which promotes calcium mobilization and protein kinase C activation. This then activates pathways leading to granule secretion, as PAR-1-stimulated Gαq-coupled ADP release is especially important for thrombin-mediated platelet activation. The Gβγ subunits can activate PI3-kinase and other lipid-modifying enzymes, protein kinases and channels. The PI3-kinase modifies the inner leaflet of the plasma membrane to provide molecular docking sites. Activation of PAR-1 can also activate growth factor shedding and activation of receptor tyrosine kinases involved in cell growth and differentiation. GEF, guanine nucleotide exchange factors; IP3, inositol triphosphate 3; PI3-kinase, phosphoinositide-3 kinase. Adapted from reference [16]

age of the receptor is potentially coupled to different G proteins that signal through multiple pathways (Figure 2) [16, 30].

Currently, four PARs have been identified, and are differentially expressed by vascular cells (endothelial cells and vascular smooth muscle cells), platelets and other circulating cells. The current family of PARs in humans consists of PAR-1, -2, -3 and -4 [31]. Of these, PAR-1, -3 and -4 are activated by thrombin, and only PAR-1 and -4 are expressed in human platelets. PAR-3 is unique in this family because it is not activated by synthetic peptides; its functional role has been demonstrated only in murine platelets, and its role in humans remains unclear [32, 33]. PAR-2 is activated by other proteases, such as trypsin and trypsinase, and coagulation factors VIIa and Xa [30, 34].

The thrombin/PAR-mediated effects in endothelial and vascular smooth muscle cells are not fully established and sometimes controversial. In fact, in normal arteries thrombin can induce endothelium-dependent vasorelaxation [35], endothelium-dependent contraction [36] or direct smooth muscle contraction [37]. Furthermore, thrombin upregulates the expression of genes related to haemostasis (for example, tissue factor, plasminogen activator inhibitor-1) and genes related to other processes (such as cell growth, angiogenesis and cytokines). Moreover, early reports showed that thrombin acts as a potent mitogen for vascular smooth muscle cells [38].

Thrombin/PAR-mediated effects in platelets are better understood. Human platelets express PAR-1 and -4, and either of them can trigger platelet secretion and aggregation [31, 39]. PAR-1 acts as the principal thrombin receptor on human platelets and mediates platelet activation by low concentrations of thrombin (EC₅₀ of 50 pM), whereas PAR-4 requires a higher concentration of thrombin (EC₅₀ of 5000 pM). PAR-3 is a unique PAR that is not activated by synthetic peptides representing its tethered peptide ligand [32, 33]. PAR-1 seems to mediate platelet activation through a hirudin-like site that acts as a thrombin-binding site, and it is responsible for the cleavage of the receptor between arginine 41 and serine 42, removing the amino-terminal sequence and generating a new protonated amino group at the NH₂-terminal of tethered ligand-S⁴²FLLRN (serine-phenylalanine-leucine-leucine-arginine-asparagine) [39]. PAR-4 lacks the hirudin-like sequence and thereby requires the higher concentrations mentioned above [40, 41]. PAR-4 uses instead a negatively charged cluster of amino-acid residues to facilitate slow disassociation from the positively charged thrombin molecule [42], and could be responsible for the prolonged effects in human platelets [43].

Thrombin-mediated PAR-1 cleavage results in the activation of heteromeric G proteins of the Gα12/13 and Gαq families that interconnect several intracellular signalling pathways to various phenotypic effects of thrombin on

platelets [21, 30] (Figure 2). These include TXA₂ production [44], ADP release [45], serotonin and adrenaline release [46, 47], activation/mobilization of P-selectin and CD40 ligand [48, 49] and platelet aggregation [50]. Moreover, this subtype of PAR stimulates platelet procoagulant activity by leading to enhanced thrombin formation [24] and the generation of fibrin from fibrinogen [51]. Furthermore, Gi-mediated signalling is stimulated by thrombin-mediated release of platelet ADP, another key pathway in platelet-mediated processes [52] (Figure 2). PAR-4 uses the same G protein subtypes [47, 53]. There are other differences between both subtypes of PAR, such as activation of PAR-4 by cathesin G [54] and a counter-regulatory mechanism of both PAR-1 and PAR-4 in angiogenesis [55]. However, the importance of these differences is not fully understood. The current hypothesis is that PAR-4 functions as an auxiliary receptor, mediating thrombin signalling to distinct effectors or with different kinetics compared with PAR-1 [16]. Other authors have suggested that PAR-1 acts as a cofactor for thrombin activation of PAR-4 on human platelets. By inhibiting the ability of thrombin to associate with PAR-1, Leger *et al.* [56] showed that PAR-1 plays a critical function in assisting PAR-4 activation by thrombin, suggesting that PAR-1 and PAR-4 exist as a complex on human platelets.

Antagonism of PAR-1

In humans, PAR-1-mediated platelet activation is one of the most important pathways in the development of pathological thrombosis; however, its role in protective haemostasis is not completely elucidated. The principal difference between normal and pathological haemostasis is the lack of propagation of the platelet-rich thrombus beyond the initial monolayer to become an occlusive clot [16]. PAR-4-deficient mice (PAR-4^{-/-}) exhibit normal platelet numbers and morphology without anaemia or spontaneous bleeding, in contrast to the severe bleeding phenotype exhibited by mice deficient in fibrinogen [57, 58]. In the PAR-4^{-/-} mice, the initial platelet accumulation at the site of injury does not differ from that in wild-type mice, and this early thrombus was proposed to support thrombin generation in sufficient amounts and allow normal fibrin deposition observed in this model [59]. In contrast, other authors have reported PAR-4^{-/-} mice to be protected against arterial thrombosis but with markedly prolonged bleeding times, suggesting that platelet activation via thrombin is required for normal haemostasis [54].

Inhibition of PAR-1 using the PAR-1 antagonist FR171113 has been shown to reduce arterial thrombosis by reducing thrombin-induced platelet aggregation without prolonging bleeding times or influencing coagulation parameters in a guinea-pig model [60]. The use of FR171113 to inhibit PAR-1 did not inhibit ADP-induced or collagen-induced platelet aggregation, suggesting that

PAR-1 antagonism does not affect other platelet signalling pathways. In the same way, the use of RWJ-58259 (PAR-1 antagonist) or antibodies to PAR-1 in primate models reduced vascular injury-induced thrombosis while sparing platelet-dependent coagulation functions, such as the activated clotting time, activated partial thromboplastin time and prothrombin times [16]. Importantly, thrombin signalling through PAR-4 remained intact in these studies, indicating a primary role of PAR-1 in mediating the prothrombotic effects of thrombin in primates. Although extrapolation of these results to humans is highly speculative, they suggest that inhibition of PAR-1 alone may be sufficient for the prevention or treatment of thrombotic events in humans. However, it cannot be excluded that preservation of thrombin signalling through platelet PAR isoforms other than PAR-1 can participate in the maintenance of normal haemostasis. These preclinical findings are also consistent with clinical observations showing substantial bleeding risk in patients treated with anticoagulants that interfere with the catalytic function of thrombin [61].

Preclinical studies

Preclinical studies have been performed with different molecules: SCH530348, SCH205831, SCH602539 and E5555. Currently, more advanced investigation is being undertaken with vorapaxar (SCH530348), currently being tested in two phase III clinical studies [62, 63], and atopaxar (E5555), which has just completed phase II testing [64–66].

Initial studies were performed in baboons and cynomolgus monkeys using the PAR-1 inhibitors SCH205831 and SCH602539, which are analogues of SCH530348. Chintala *et al.* [67], using a cynomolgus monkey model, showed that oral administration of SCH205831 caused a complete and dose-dependent inhibition of thrombin/PAR-induced platelet aggregation until 6 h after dosing. This PAR-1 inhibitor administered orally at a dose of 10 mg kg⁻¹ was able to inhibit platelet deposition by 50–70% onto uncoated stents in an arteriovenous shunt model of thrombosis in baboons [67]. Bleeding times, coagulation parameters and ADP-induced platelet aggregation were unchanged by the use of this drug in this model. The same group showed the efficacy of another selective and orally active PAR-1 inhibitor, SCH602539, in a dose-dependent manner using the Folts model of thrombosis in anaesthetized cynomolgus monkeys [68].

Vorapaxar (SCH530348)

Following the studies of the above-mentioned analogues, SCH530348, currently named vorapaxar, was selected for further clinical development. Vorapaxar was originally developed by Schering Plough, which has now been acquired by Merck pharmaceuticals. This PAR-1 inhibitor was shown to completely inhibit thrombin receptor

agonist peptide (TRAP)-induced platelet aggregation with oral administration at doses $\geq 0.1 \text{ mg kg}^{-1}$ for 24 h, in animal models (cynomolgus monkeys). In an animal model, vorapaxar showed a bioavailability of 86% [69]. In human platelet-rich plasma, vorapaxar inhibited thrombin and TRAP-induced platelet aggregation with an IC_{50} of 47 and 25 nM, respectively, without affecting aggregation induced by other reagents, such as ADP, TXA2 mimetic U46619 or collagen. However, and more importantly, vorapaxar did not affect prothrombin time or activated partial thromboplastin time, suggesting that the potential for bleeding events may not be increased [69]. These results are in agreement with the observations derived from cynomolgus monkeys that treatment with vorapaxar either alone or in combination with aspirin plus clopidogrel did not increase bleeding time or surgical blood loss compared with placebo [70]. On the basis of these promising results, vorapaxar was advanced into clinical development.

Pharmacological aspects

Vorapaxar is a synthetic tricyclic 3-phenylpyridine analogue of himbacine, a natural product that has been modified as a crystalline salt for drug development and clinical use (Figure 3) [71, 72]. Vorapaxar is a nonprotein, small-molecule, high-affinity, orally active, competitive PAR-1 inhibitor [71, 72]. It is a potent competitive antagonist of the platelet thrombin receptor PAR-1 (K_i of 2.7 nmol l^{-1}), blocking thrombin-mediated platelet activation without interfering with cleavage of fibrinogen, the final step of coagulation, which is also provided by thrombin. In kinetic studies, this molecule showed a long receptor dissociation half-life of approximately 20 h. It is rapidly absorbed, presenting a slow elimination with a calculated half-life up to

311 h. The routes of elimination are mainly faeces and secondarily by renal clearance (less than 5%) [29]. Exposure to vorapaxar is dose related, with an interindividual variability in the range of 20–40% and without any significant difference in exposure among sex and race in fasting conditions [62]. The co-administration of food does not affect the overall drug exposure; however, time after administration when the maximum plasma concentration is reached (T_{max}) and maximum plasma concentration (C_{max}) are slightly delayed, and therefore, maximal platelet inhibition is delayed by approximately 2 h. Vorapaxar is slowly but extensively metabolized by cytochrome P450 (CYP) 3A4. Therefore co-administration of drugs that modify the metabolic activity of CYP3A4 could potentially modulate vorapaxar exposure to platelets. Some evidence has been reported for ketoconazole, a potent CYP3A4 inhibitor, and rifampin, a potent CYP3A4 inducer, which may, respectively, increase or reduce, after a period of co-administration longer than 3 weeks, drug exposure for about the half of the theoretical value (Table 1) [62, 63, 73].

Phase I studies

The first phase I study using vorapaxar was conducted in 50 healthy volunteers taking a single oral 5–40 mg dose [74]. This regimen was able to produce >90% mean inhibition of TRAP-induced platelet aggregation in all subjects for >72 h, with low interindividual variability in plasma concentration. In this study, vorapaxar was characterized by rapid absorption and slow elimination, with a half-life of >72 h.

Another phase I study demonstrated that a 40 mg single dose of vorapaxar was safe and well tolerated in healthy Caucasian and Japanese subjects [75, 76]. In both populations, 80% or more inhibition of TRAP-induced

Vorapaxar
(SCH530348)

Atopaxar
(E5555)

Figure 3

Chemical structure of vorapaxar (SCH530348) and atopaxar (E5555)

Table 1

Characteristics of vorapaxar (SCH530348) and atopaxar (E5555)

	Vorapaxar	Atopaxar
Formulation	Oral	Oral
Mechanism of action	Competitive and reversible	Competitive and reversible
Onset	~2 h	~3.5 h
Half-life	311 h	23 h
IC₅₀ to inhibit thrombin-induced platelet aggregation	47 nM	64 nM
Main metabolic activation	CYP3A4	CYP3A4
Major route of elimination	Faeces	Faeces
Development status	Phase III	Phase II

Adapted from reference [29].

platelet aggregation was achieved rapidly and consistently with this drug regimen. In a separate, recently reported phase I study in healthy subjects, the dosing regimen of vorapaxar that most consistently provided the targeted level of TRAP-induced platelet inhibition throughout the entire 28 day treatment period was a 40 mg loading dose followed by a 2.5 mg once-daily maintenance dose [77].

Phase II studies

The safety profile of vorapaxar was evaluated in a multi-centre, large, randomized, double-blind, placebo-controlled, dose-ranging clinical trial, the Thrombin Receptor Antagonist for Cardiovascular Event Reduction in Percutaneous Coronary Intervention (TRA-PCI) trial, which enrolled 1030 patients in 76 centres [78]. This trial evaluated the safety and efficacy of vorapaxar used in combination with standard oral antiplatelet therapy (aspirin and clopidogrel) and an antithrombotic agent (heparin or bivalirudin) over a treatment duration of 60 days in patients undergoing non-urgent PCI or coronary angiography with planned PCI. The study design was a 3:1 model randomization to receive placebo or vorapaxar on top of standard-of-care antithrombotic therapy, including aspirin, clopidogrel and an anticoagulant of choice. Patients assigned to vorapaxar received one of three single loading doses (10, 20 or 40 mg) before angiography and a maintenance therapy with one of three doses (0.5, 1.0 or 2.0 mg daily) for 2 months in the 56% of patients who underwent PCI. The primary end-point was the incidence of the composite of thrombolysis in myocardial infarction (TIMI) major or minor bleeding in the PCI cohort. No differences were observed in terms of primary end-point between vorapaxar (with all loading and maintenance doses) and placebo groups. No differences in the incidence of TIMI major and minor bleeding were also observed, although a trend towards increased bleeding rates with higher doses was observed (Tables 2 and 3). Interestingly, although the study was not powered to evaluate efficacy end-points, a

strong trend was observed among patients treated with vorapaxar towards a lower rate of the composite end-point of death and major adverse cardiac events, including myocardial infarction (placebo 9% vs. vorapaxar 6%; absolute difference -2.9, 95% confidence interval -7.9 to 2.1). Vorapaxar also demonstrated a dose-dependent inhibition of TRAP-induced platelet aggregation, inhibiting almost 30% of patients as early as 0.5 h with a 40 mg loading dose. This effect was substained over 60 days with each maintenance dose [79].

These results were confirmed in two small randomized trials conducted in Japanese patients: one performed in non-ST elevation acute coronary syndrome (NSTEMI-ACS) patients undergoing PCI ($n = 117$) [80], and the other among patients with previous ischaemic stroke ($n = 90$) [81]. Both studies showed a good safety profile, in terms of no increase in TIMI major and minor bleeding using different vorapaxar dosages (20 or 40 mg loading dose, followed by 1 or 2.5 mg daily maintenance dose in NSTEMI-ACS patients, and 1 or 2.5 mg daily maintenance dose for 60 days in patients with ischaemic stroke). In the first study, conducted in NSTEMI-ACS patients, treatment with vorapaxar was also associated with a significant reduction of periprocedural myocardial infarction [80].

Phase III studies

The favourable results derived from phase II testing led to the design of the following two large phase III clinical trials, which are currently ongoing: the Thrombin Receptor Antagonist for Clinical Event Reduction in ACS (TRACER) [62] and Thrombin Receptor Antagonist in Secondary Prevention of Atherothrombotic Ischemic Events (TRA 2P-TIMI 50) [63] trials. These trials have been designed and powered to assess the efficacy of vorapaxar to reduce recurrent ischaemic events. However, these trials will be very important to assess the safety of vorapaxar, in particular its impact on bleeding. In fact, while phase II testing provided reassuring safety information, which allowed expansion into phase III testing, these studies are too limited in sample size to fully determine the impact of this drug on bleeding complications, which remain a major concern in the current era of antithrombotic agents. In fact, it is important to underscore the emerging data concerning the prognostic implications of bleeding, including effects on mortality, which emphasize the importance of the balance between safety and efficacy of antithrombotic agents [82].

The TRACER trial [62] is a prospective, randomized, double-blind, multicentre trial designed to assess whether the use of vorapaxar added to standard-of-care antiplatelet treatment (aspirin plus clopidogrel) will reduce the incidence of a composite end-point consisting of cardiovascular death, myocardial infarction (MI), stroke, recurrent ischaemia with rehospitalization and urgent coronary revascularization in patients admitted with non-ST elevation ACS. This study is planned to enrol

Table 2

The results of the Thrombin Receptor Antagonist for Cardiovascular Event Reduction in Percutaneous Coronary Intervention (TRA-PCI) trial by loading dose

Safety and efficiency outcomes		Vorapaxar by loading dose				
		Placebo	All doses	10 mg	20 mg	40 mg
PCI	Number of patients	151	422	129	120	173
	TIMI major/minor bleeding	5 (3.3%)	12 (2.8%)	2 (1.6%)	3 (2.5%)	7 (4.0%)
	TIMI major bleeding	2 (1.3%)	3 (0.7%)	2 (1.6%)	0	1 (0.6%)
	TIMI minor bleeding	3 (2.0%)	9 (2.1%)	0	3 (2.5%)	6 (3.5%)
	Death/MACE	13 (8.6%)	24 (5.7%)	10 (7.8%)	6 (5.0%)	8 (4.6%)
	Myocardial infarction	11 (7.3%)	18 (4.3%)	7 (5.4%)	5 (4.2%)	6 (3.5%)
CABG	Number of patients	24	52	10	18	24
	TIMI major / minor bleeding	19 (79%)	47 (90%)	9 (90%)	17 (94%)	21 (88%)
	TIMI major bleeding	9 (38%)	33 (63%)	8 (80%)	12 (67%)	13 (54%)
	TIMI minor bleeding	10 (42%)	15 (29%)	1 (10%)	5 (28%)	9 (38%)
	Chest tube drainage (ml)*	996 (516–1724)	988 (690–1750)	1393 (975–1750)	1015 (690–1670)	870 (414–1790)
	PRBC transfusion (>2 units)	5 (21%)	9 (17%)	2 (20%)	2 (11%)	5 (21%)

*Median (interquartile). Abbreviations: CABG, coronary artery bypass graft; MACE, major adverse cardiac event; PCI, percutaneous coronary intervention; PRBC, packed red blood cells; TIMI, thrombolysis in myocardial infarction. Adapted from reference [79].

Table 3

Rate of bleeding events in the Thrombin Receptor Antagonist for Cardiovascular Event Reduction in Percutaneous Coronary Intervention (TRA-PCI) trial by maintenance dose

	Placebo	Vorapaxar by maintenance dose		
		0.5 mg	1.0 mg	2.5 mg
Number of patients	63	136	139	138
TIMI major/minor bleeding	1 (2%)	3 (2%)	5 (4%)	4 (3%)
TIMI major bleeding	0	0	2 (1%)	1 (<1%)
TIMI minor bleeding	1 (2%)	3 (2%)	3 (2%)	3 (2%)

Abbreviation: TIMI, thrombolysis in myocardial infarction. Adapted from reference [79].

approximately 10 000 patients, who will be randomized to vorapaxar (40 mg loading dose and 2.5 mg daily maintaining dose) for at least 1 year vs. placebo. This study is also designed to assess the safety of the use of vorapaxar, defined as incidence of moderate and severe bleeding events according to the Global Use of Strategies to Open Coronary Arteries (GUSTO) classification, and clinically significant bleeding, defined as TIMI major or TIMI minor bleeding, or bleeding that requires unplanned medical treatment, surgical treatment or laboratory evaluation even if not meeting the criteria for TIMI major or TIMI minor bleeding [62].

The TRA 2P-TIMI 50 trial [63] is a prospective, randomized, double-blind, multinational trial randomizing patients to vorapaxar administered only as a 2.5 mg daily maintenance dose vs. placebo in addition to current standard-of-care. This study is being conducted in patients with history/evidence of atherosclerosis involving the coronary, cerebral or peripheral vascular systems (secondary prevention). The study has completed enrollment of approxi-

mately 27 000 patients. The primary end-point is the composite of the first occurrence of any component of the composite of cardiovascular death, MI, stroke and urgent coronary revascularization, during 1 year of follow-up. Safety end-points, in particular bleeding, are also being evaluated [63].

Atopaxar (E5555)

Atopaxar, developed by Eisai pharmaceuticals, is the other PAR-1 antagonist which has completed phase II clinical development. Preclinical studies of atopaxar showed anti-thrombotic effects in animal models (guinea-pigs) [83]. Furthermore, atopaxar was shown to have inhibitory effects on neointimal hyperplasia in a rat balloon injury model [79] and the release of the inflammatory markers soluble CD40 ligand (sCD40L) and interleukin-6 and expression of P-selectin [83]. Moreover, this compound was also able to show inhibitory effects on thrombin-induced contractile response (spasm) in a rabbit subarachnoid haemorrhage model [83, 84]. In healthy human volunteers, atopaxar was shown to inhibit thrombin- and TRAP-mediated CD40L release (IC₅₀ 47 and 38 nM, respectively) without affecting ADP-induced CD40L release [84].

Pharmacological aspects

Atopaxar is an orally active and potent PAR-1 antagonist that specifically targets the G protein-coupled receptor and binds to PAR-1 at the tethered ligand binding site on platelet membranes [29]. It is a small molecule, chemically identified as 1-(3-tert-butyl-4-methoxy-5-morpholinophenyl)-2-(5,6-diethoxy-7-fluoro-1-imino-1,3-dihydro-2H-isoindol-2-yl) ethanone hydrobromide, with a molecular weight of 608.54 Da (Figure 3). The drug inhibits the binding of high-

affinity [^3H]-TRAP, presumably at or near the tethered ligand-binding epitope site [85]. Atopaxar has a slower onset of its effects (3.5 h) and lower half-life (23 h) in compared with vorapaxar. Like vorapaxar, atopaxar is mainly metabolized by CYP3A4, and its major route of elimination is faeces (Table 1) [29].

Phase I studies

The effects of escalating concentrations of atopaxar (20, 50 and 100 ng ml⁻¹) on platelet function were evaluated *in vitro* in healthy volunteers and coronary artery disease (CAD) patients with or without clopidogrel, by Serebruany *et al.* [86]. Atopaxar achieves almost complete inhibition of TRAP-induced platelet aggregation, with a very minor impact on ADP- or collagen-induced aggregation (10–15% inhibition of 5 μM ml⁻¹ ADP- or 1 μM ml⁻¹ collagen-induced platelet aggregation in plasma). Platelet inhibition was present at a concentration of 20 ng ml⁻¹ and was not dose dependent, and a further increase of inhibition was not observed after incubation with 50 and 100 ng ml⁻¹. However, these effects were not shown in whole blood. Moreover, platelet biomarkers were also significantly reduced.

The pharmacodynamics and safety of oral administration were evaluated in two studies on healthy volunteers. Marion *et al.* [87] assessed the effects of ascending oral doses of 50, 100 and 200 mg in a randomized, double-blind and placebo-controlled manner. Doses of 100 and 200 mg achieved a similar maximum plasma concentration and area under the curve, with 91 and 95% platelet inhibition, respectively. Takeuchi *et al.* [88], in a similar way, found an 80% or more thrombin-induced platelet inhibition with a single dose of 50 mg orally. Repeated administration of 100 and 200 mg was associated with almost 100% platelet inhibition. There were no severe adverse events reported in either study.

Phase II studies

The results of phase II studies have been reported recently. The J-LANCELOT (Japanese-lesson from antagonizing the cellular effect of thrombin) trial [64] comprises two double-blind, placebo-controlled phase II studies conducted in ACS patients and in high-risk CAD patients. The ACS study randomized 241 patients with NSTEMI-ACS into four groups (placebo, 50, 100 and 200 mg atopaxar), receiving the drug daily for 12 weeks. Patients randomized to any dose of atopaxar also received a 400 mg loading dose (vs. a placebo loading dose). The CAD study randomized 263 patients into the same groups as in the ACS study, but without giving a loading dose and with a 24 week follow-up. The primary end-point was the incidence of bleeding events, classified according to the Clopidogrel in Unstable Angina to Prevent Recurrent Ischemic Events (CURE) (major and minor bleeding) and TIMI (major, minor and minimal bleeding) definitions. The secondary end-point was the incidence of major cardiovascular adverse events

(MACEs), such as cardiovascular death, MI, stroke or recurrent ischaemia. In addition, TRAP-induced aggregation was performed using light transmittance aggregometry following 15 μM TRAP stimuli. Briefly, there was a low incidence of CURE bleeding in both groups of patients, without achieving any statistical significance. The entire population showed the same tendency when the events were classified according to the TIMI definition. However, there was a numerical increase in any TIMI bleeding with the dose of 200 mg (ACS study, 16.4% placebo vs. 23.0% atopaxar, $P = 0.398$; and CAD study, 4.5% placebo vs. 13.2% atopaxar, $P = 0.081$). No significant dose-dependent difference was observed in ACS patients for the incidence of TIMI bleeding ($P = 0.266$) and no significant difference was observed between placebo and all combined groups ($P = 0.609$). However, a dose-dependent trend was observed in the CAD study ($P = 0.086$), without significant differences between placebo and treated groups ($P = 0.219$). Interestingly, no patient experienced TIMI major bleeding in any group. Regarding the secondary end-point, there was a numerical reduction in MACEs among patients treated with 100 and 200 mg atopaxar. However, MACE rates in both treatment and placebo groups was not significantly different (5.0% placebo vs. 6.6% atopaxar, relative risk 0.76, 95% confidence interval 0.257–2.606, $P = 0.73$ in the ACS study; and 4.5% placebo vs. 1.0% atopaxar, relative risk 0.22, 95% confidence interval 0.041–1.109 in the CAD study). The most frequent MACE was recurrent ischaemia, without any cardiovascular death in both groups. The pharmacodynamic assessment of both studies (ACS, 42 patients and CAD, 80 patients) showed 20–50% platelet inhibition in the ACS study and almost 50% inhibition in the CAD study with a 50 mg daily maintenance dose of atopaxar. Using 100 or 200 mg, more than 90% inhibition was observed in both studies. In the ACS study, the mean platelet aggregation inhibition reached >80% at 3–6 h after administration of the 400 mg loading dose of atopaxar. These results are in agreement with previous phase I studies. An interesting finding of these studies was the more frequent treatment-related adverse events in patients treated with atopaxar than in patients receiving placebo (44.4 vs. 27.9%, respectively, in ACS patients, $P = 0.024$; 32.0 vs. 13.6%, respectively, in CAD patients, $P = 0.003$). In both studies, the most common adverse event related to the study drug was abnormal liver function, with a rate of around 12–15% in patients treated with 200 mg of the study drug in both studies. It has been suggested that liver function abnormalities may be attributed to concomitant thienopyridine use. There were also dose-related effects on QT corrected interval (QTc) noted with atopaxar, showing an interval prolongation with increasing doses.

Most recently, the LANCELOT ACS and CAD studies, conducted outside Japan, have been presented [65, 66]. These studies had the same design and primary end-point as J-LANCELOT. The LANCELOT ACS and CAD trials enrolled 603 and 720 patients, respectively. There was no statistical

difference in the primary end-point between placebo and atopaxar in both studies. There was no difference in any TIMI bleeding event in LANCELOT ACS (placebo 10.1%, atopaxar total 9.3%, relative risk 0.91, $P = 0.77$). There was a trend to more TIMI bleeding (placebo 6.8%, atopaxar total 10.3%, relative risk 1.52, $P = 0.17$) and CURE minor bleeding events (placebo 0.6% vs. atopaxar 3.0%, relative risk 5.2, $P = 0.08$) in LANCELOT CAD. In LANCELOT ACS, patients receiving 100 mg of atopaxar showed a nonstatistical increase of bleeding events (both CURE and TIMI). No differences in the primary efficacy end-point were observed (7.8% in placebo vs. 8.0% in atopaxar group; $P = 0.93$); however, patients under atopaxar treatment showed less incidence of Holter-ECG-detected ischaemia (28.1% in placebo vs. 18.7% in atopaxar group; $P = 0.02$) among ACS patients. Moreover, in LANCELOT CAD, patients treated with atopaxar showed a numerically, but not statistically lower incidence of events, which did not appear to be dose related (placebo 4.6%, atopaxar total 2.6%, relative risk 0.57, $P = 0.20$). With regard to adverse events, the presence of liver dysfunction was observed only in 5.47% of patients on 200 mg of atopaxar among ACS patients. However, only three of these patients discontinued the drug. Overall, prolongation of QTc was seen in all study arms from randomization to end of treatment, being more exacerbated with doses of 100 and 200 mg, without any case of syncope or malignant arrhythmias. Regarding CAD patients, this transient elevation in liver transaminases and dose-dependent QTc prolongation without apparent complications were also observed in higher dose atopaxar treatment groups. Pharmacodynamic results of LANCELOT ($n = 39$ ACS and 80 CAD patients) corroborate the J-LANCELOT findings. Atopaxar achieved up to 92% of TRAP-induced platelet aggregation inhibition at 3–6 h after the loading dose, and 92% with a 200 mg maintenance dose at the 8th week of treatment in the ACS population, or 99% with the same dose at 12 weeks in the CAD population.

Plans for phase III development with atopaxar are not fully determined at the present time.

Perspectives and novel approaches with PAR antagonism

Although vorapaxar and atopaxar have similar pharmacological actions, there are differences between these two compounds. In particular, vorapaxar has a longer half-life than atopaxar. This property makes atopaxar more suitable for patients who may need rapid suspension of drug effects (e.g. in case of surgery). However, phase II testing suggests that atopaxar is associated with more liver dysfunction. Overall, although both drugs showed an increase in bleeding with higher doses, they demonstrated a safety bleeding risk profile when used in combination with current standard therapy (aspirin and a thienopyridine). Importantly, inhibition of the PAR-1 does not alter normal

homeostasis, which is key in protecting against blood loss. This is in contrast to P2Y₁₂ inhibition strategies, which can modulate thrombin generation profiles [89] and are associated with increased bleeding events when more potent strategies are used [11, 12, 90]. Overall, the pivotal role of thrombin-mediated platelet activation in atherothrombotic processes and the properties of PAR-1 antagonists have also led to suggestions regarding the potential for these agents to be considered not only as an 'add-on' therapy but also as an 'alternative' therapy to current standard-of-care. Indeed, future investigations will better elucidate these therapeutic possibilities.

In addition to the established antithrombotic effects of PAR-1 antagonists, other properties appear to be of potential interest. Among these, their effects on smooth muscle cell contraction are of particular interest [91]. In an animal model, Bocquet *et al.* [92] demonstrated that a new PAR-1 antagonist, F121958, is a potent antivasoconstrictor, due to its properties in smooth muscle cells, among lesioned vessels. Moreover, this PAR-1 inhibitor did not have haemodynamic effects in normal vasculature. This shows the beneficial effect of PAR-1 inhibitors to prevent vasospasm caused by arterial injury and mediated by thrombin, in addition to their antithrombotic properties. Another interesting property is the synergistic effect on inhibition of different activation pathways. Chintala *et al.* [93] recently reported that the combined inhibition, in an animal model, with SCH602639 (PAR-1 antagonism) and cangrelor (P2Y₁₂ inhibitor) was associated with synergistic antithrombotic effects in comparison with vehicle for all combinations tested in a Folts model. Other potential targets associated with PAR-1 antagonism are currently under investigation.

Conclusion

Platelets have an important role in normal haemostatic processes, but they also mediate pathological thrombosis. Multiple pathways contribute to platelet activation. Among these, thrombin-mediated platelet activation through PAR-1 signalling has a key role. Inhibition of the PAR-1 receptor is the target for novel promising antiplatelet drugs. Vorapaxar (SCH530348) and atopaxar (E5555) are PAR-1 antagonists which have completed phase II testing, showing overall favourable safety profiles and encouraging clinical outcomes (Table 4). Larger scale clinical trials will better define the efficacy and safety of PAR-1 antagonists in addition to standard-of-care in patients with atherothrombotic disease manifestations.

Competing Interests

Dominick J. Angiolillo reports receiving the following: honoraria for lectures from Bristol Myers Squibb, Sanofi-Aventis, Eli Lilly Co. and Daiichi Sankyo, Inc.; consulting fees

Table 4
Clinical trials with vorapaxar and atopaxar

Drug	Trial/study author	Phase	Type	Patients	n	Dose of drug	Primary end-point	Follow-up	Status	Major finding
Vorapaxar	TRA-PCI [75]	II	Randomized, multicentre, double-blind, placebo controlled	Elective PCI	1030	10–20–40 mg (LD) 0.5–1–2.5 mg (MD)	TIMI major and minor bleeding	60 days	Completed	No difference in primary end-point
	Goto <i>et al.</i> [77]	II	Randomized, single-centre, placebo controlled	NSTEMI	117	20–40 mg (LD) 1–2.5 mg (MD)	TIMI major and minor bleeding	60 days	Completed	No difference in primary end-point; less MI in treatment arm
	Shinohara <i>et al.</i> [78]	II	Randomized, single-centre placebo controlled	Previous stroke	90	1–2.5 mg (MD)	TIMI major and minor bleeding	60 days	Completed	No difference in primary end-point
	TRA-CER [54]	III	Randomized, multicentre, double-blind, placebo controlled	ACS	10 000	40 mg (LD) 2.5 mg (MD)	Death, stroke, MI and TLR	1 year	Recruiting	–
	TRA 2P-TIMI 50 [60]	III	Randomized, multicentre, double-blind, placebo controlled	History of atherosclerosis	26 000	2.5 mg (MD)	Death, stroke, MI and revascularization	Anticipated average duration of 24 months (at least 1 year)	Active, not recruiting	–
Atopaxar	J-LANCELOT [61]	II	Two randomized, single-centre, placebo controlled	ACS CAD	241 263	400 mg (LD); 50– 100–200 mg (MD); 50–100–200 mg (MD)	TIMI and CURE major and minor bleeding	12 weeks 24 weeks	Completed	No difference in primary end-point, although a higher rate of any TIMI bleeding with higher doses; most common adverse event abnormal liver function
	LANCELOT-ACS [62]	II	Randomized, multicentre, double-blind, placebo controlled	ACS	603	400 mg (LD); 50– 100–200 mg (MD)	TIMI major and minor bleeding	12 weeks	Completed	No difference in primary end-point; transient elevation of liver enzymes and QTcF prolongation with higher doses
	LANCELOT-CAD [63]	II	Randomized, multicentre, double-blind, placebo controlled	CAD	720	50–100–200 mg (MD)	TIMI major and minor bleeding	24 weeks	Completed	No difference in primary end-point, although a higher rate of any TIMI bleeding with higher doses; transient elevation of liver enzymes and QTcF prolongation with higher doses

Abbreviations: ACS, acute coronary syndrome; CAD, coronary artery disease; LD, loading dose; MD, maintenance dose; MI, myocardial infarction; NSTEMI, non-ST elevation myocardial infarction; PCI, percutaneous coronary intervention; TIMI, thrombolysis in myocardial infarction; TLR, target lesion revascularization; QTcF, corrected QT interval (Framingham); CURE, The clopidogrel in unstable angia to prevent recurrent events.

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The other authors have no competing interests to declare.

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