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INVITED REVIEW

Antiplatelet drugs: which targets for which treatments?

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Summary. The current standard care for acute coronary syndromes is dual antiplatelet therapy combining the COX1 inhibitor aspirin with a drug targeting the $P2Y_{12}$ receptor, together with anticoagulation during and after early revascularization by percutaneous intervention. In very high-risk patients, glycoprotein (GP) IIb/IIIa antagonists may also be used. Secondary prevention of ischemic events requires dual antiplatelet therapy for several months followed by lifelong low-dose aspirin. The duration of treatment and the drugs to combine nevertheless remain matters of debate and the focus of ongoing research. Despite great progress, there is still room for improved efficacy and this could involve new targets for both antiplatelet drugs (like the thrombin receptor PAR1) and anticoagulants. However, improved efficacy is offset by an increased risk of bleeding. Stroke patients are still waiting for better treatment, their bleeding risk being particularly high. New targets including the collagen receptor, glycoprotein VI (GPVI), and the GPIb-von Willebrand factor axis, governing platelet interaction with the diseased vessel wall, should enable us to complete the armamentarium of antiplatelet drugs.

Keywords: aspirin; clopidogrel; coronary artery disease; platelets; stroke; thrombosis.

Introduction

Antiplatelet drugs are used for the treatment and the secondary prevention of ischemic diseases of the arterial vascular bed, which include coronary artery disease (CAD), cerebrovascular stroke, and peripheral arterial disease (PAD). CAD includes stable angina and acute coronary syndromes (ACS), which are further divided into unstable angina, non-ST-elevation myocardial infarction (NSTEMI), and ST-elevation myocardial infarction (STEMI). Depending on the type and severity of the arterial disease, the standard care for thrombotic episodes involves either a pharmacological approach alone, revascularization by percutaneous coronary intervention (PCI), or surgical placement of a vascular bypass graft. In all cases, various regimens of antiplatelet drugs are used to prevent reocclusion. Numerous guidelines exist to help clinicians to decide how to manage the various situations they encounter.

Before the late 1990s, the question of the best targets for antiplatelet drugs would not have made sense, as the only existing drugs were aspirin, used as an antithrombotic drug since the mid-sixties, and ticlopidine, discovered in the early seventies and marketed in 1978 without any knowledge of its mechanism of action [1,2]. The mechanism of action of aspirin was elucidated in the seventies. At the same time, the first-generation thienopyridine ticlopidine and the second-generation thienopyridine clopidogrel were used as molecular tools to unravel the pathways of ADP-induced platelet activation and the central role of ADP in the mechanisms of thrombus formation *in vivo* [3]. Since then, great progress has been made in our understanding of platelet physiology and the involvement of platelets in thrombosis. The late 1980s and 1990s were decades in which firstly, the major platelet membrane glycoproteins (including the $\alpha_{IIb}\beta_3$ integrin, the GPIb-V-IX complex, and GPVI [4,5]) and secondly, the G protein-coupled receptors for most known platelet agonists (including thrombin, ADP, thromboxane A_2 [TXA_2], adrenalin, and serotonin) were cloned and sequenced [6]. This opened up the way for the use of parallel approaches to identify the targets of existing or candidate drugs. Concomitantly, rational drug design and high-throughput screening of large pharmacochemical libraries allowed the discovery of compounds acting on new potential antithrombotic targets [7].

Antiplatelet drugs should be of proven efficacy in the secondary prevention of ischemic events without incurring an unacceptable increase in the risk of bleeding [8]. Long-term therapy should be safe as premature cessation of the medication or non-compliance, often due to bleeding, can result in the recurrence of ischemic events [8]. One may draw up a scale of increasing bleeding risk for antiplatelet targets and their roles in platelet functions, where the $\alpha_{IIb}\beta_3$ integrin would lie at the right edge and the collagen receptor GPVI or the purinergic receptors $P2X_1$ and $P2Y_1$ at the left edge, with intermediate positions for

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targets such as the TP receptors for TXA_2, cyclooxygenase 1 (COX1), phosphatidylinositide-3-kinases (PI3K), and other intracellular or membrane targets, the list being very long. On such a risk scale, the $P2Y_{12}$ receptor would lie very close to the $\alpha_{IIb}\beta_3$ integrin (Fig. 1).

Theoretically, when the thrombotic risk is high, the antiplatelet therapy should achieve strong inhibition of platelet function. Conversely, if the risk lies more on the bleeding side, the treatment should be more conservative. This can be difficult as the patients who bleed also appear to be those whose thrombotic risk is the highest. Moreover, one has to distinguish between the acute phase of CAD and PCI, a thrombogenic gesture by nature, which both require strong antithrombotic treatment targeting simultaneously platelet activation and thrombin generation, and the maintenance phase of therapy where the bleeding risk must be tightly controlled [8–10].

The relevance and quality of a potential target for antiplatelet drugs are related to its role in the mechanisms of thrombus formation and for some of them, the fact that they are exclusively expressed on platelets. This is clearly the case for the $\alpha_{IIb}\beta_3$ integrin and for GPVI. Interestingly, the $P2Y_{12}$ ADP receptor was long thought to be expressed only on platelets and was previously termed P2T, where T stood for 'thrombocyte' [3]. On the other hand, the mechanism of action of the drug, drug dosage, right combination of drugs, duration of treatment, clinical setting, and procedure have their own impact on the overall outcome, independently of the quality of the target.

Distinct progress has been made over the last 15 years in the treatment of CAD. Nonetheless, there is still high mortality and morbidity in these patients in the long term [11]. Moreover, stroke patients receive inadequate support because there is a definite lack of drugs, which can be used to efficiently inhibit ischemia while avoiding cerebrovascular bleeding [12]. Finally, patients with PAD are often overlooked and once again, we lack good pharmacological protocols [13]. Because of all these limitations, there is still an important need for new molecules possibly acting on new targets in addition to the existing drugs.

Currently, the principal antiplatelet targets are COX1, targeted by aspirin, the $P2Y_{12}$ receptor targeted by the thienopyridine compounds and by direct antagonists including ticagrelor and cangrelor, and the $\alpha_{IIb}\beta_3$ integrin targeted by the so-called GPIIb/IIIa inhibitors. Besides these well-established targets, intense clinical research is devoted to the evaluation of new drugs and their platelet targets, among which the most promising are the thrombin receptor PAR1, the platelet-specific collagen receptor GPVI, and possibly, the GPIb-von Willebrand factor (VWF) axis. In addition, numerous potential targets are under preclinical investigation, including PI3K, Syk, and other integrins, but this topic would be beyond the scope of this short review.

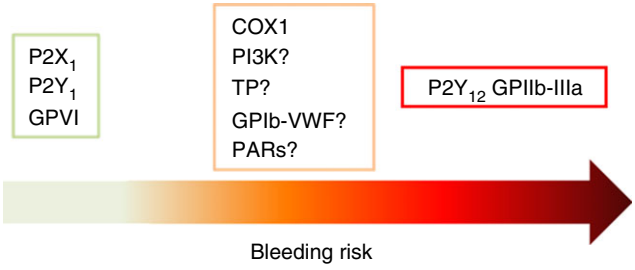

Fig. 1. Pharmacological targets for antiplatelet drugs and the bleeding risk.

Aspirin and COX1 inhibition

Aspirin is the oldest antiplatelet drug. Synthesized in the late nineteenth century, its antiplatelet effects were observed in the mid-1960s and its molecular mechanisms of action discovered during the 1970s [1,7]. In 1971, John Vane found that aspirin inhibited prostaglandin synthesis. In fact, aspirin irreversibly inactivates COX1. COX1 catalyzes the production of prostaglandin H_2 (PGH_2) from arachidonic acid (AA), which is released from membrane phospholipids by phospholipase A_2 (PLA_2) during cell activation. In platelets, PGH_2 is then transformed into TXA_2 through the action of TXA_2 synthase. TXA_2 formation occurs in response to the platelet activation triggered by various agonists including thrombin and collagen and is one of the amplification loops, which strengthen platelet aggregation and contribute to the recruitment of circulating platelets. Moreover, TXA_2 is a potent vasoconstrictor.

The effect of aspirin is irreversible, which means that a single daily dose of 75–100 mg is sufficient to completely inhibit TXA_2 formation in platelets. In subjects, diabetic or non-diabetic, whose cyclooxygenase recovery is accelerated, or in patients with myeloproliferative diseases such as polycythemia vera, low-dose aspirin twice a day has been proposed to be a good way to correct the apparent lower responsiveness to the drug [14,15]. On the other hand, the bleeding risk of aspirin is correlated with both its antiplatelet effect and its inhibition of the gastrointestinal production of protective prostaglandins. Therefore, the bleeding risk of aspirin increases as the dose increases, independently of its impact on platelets [1].

Depending on the patient's profile, aspirin has a net beneficial effect or its benefit can be offset by the occurrence of gastrointestinal and/or intracranial bleeding. In primary prevention trials, a 12% reduction in the risk of serious vascular events was observed, mainly due to the reduced occurrence of non-fatal myocardial infarction. In these studies, no effect on stroke was observed as the small antithrombotic benefit was counterbalanced by increased bleeding [16]. In the case of secondary prevention, considering various clinical trials and meta-analyses, low-dose aspirin (75–100 mg day^{-1}) reduced the occur-

rence of ischemic events by 20%, resulting in 15 fewer vascular events per 1000 patients treated [17].

Thus, the net benefit of aspirin increases when the thrombotic risk is high while in primary prevention, the risk of bleeding is equivalent and one may question the relevance of its use. This is still a subject of debate and various clinical trials are in progress in an attempt to answer specific questions such as the influence of patient subpopulations, age, and comorbidities like diabetes [18]. Concerning specifically ischemic stroke and transient ischemic attack (TIA), early and prolonged administration of aspirin is recommended [17,19,20]. Aspirin combined with dipyridamole is also recommended. A recent meta-analysis concluded that short-term dual antiplatelet therapy is efficient and safe, whereas long-term dual therapy increases the risk of major and intracranial bleeding [21]. However, as the MATCH study did not find any additional beneficial effect of combined aspirin and clopidogrel as compared to clopidogrel alone, many patients are still treated with either aspirin alone or clopidogrel alone [12,22]. In patients with PAD, aspirin seems to be associated with a poor outcome and there is a lack of studies comparing aspirin with more efficient antiplatelet regimens [13].

TP receptors and TXA_2 synthase

TP receptors are G protein-coupled receptors expressed on platelets and on various inflammatory cells in blood and the vascular wall, namely macrophages, monocytes, endothelial cells, and smooth muscle cells. Theoretically, these receptors should be more relevant antithrombotic targets as blockade of only COX1 by aspirin can be overcome by other ligands such as prostaglandins, endoperoxides, and isoprostanes, or by transcellular processing of other sources of endoperoxides to produce TXA_2 [23]. Thus, blocking the enzyme forming the real agonist and/or the receptors mediating its effects should be more effective. However, although several drugs including terutroban, a TP receptor antagonist, or picotamide, a dual TP receptor antagonist and TXA_2 inhibitor, have displayed promising profiles *in vitro* and in animal models, they showed no superiority to aspirin in a clinical setting [24] except perhaps in patients with PAD [25].

The $P2Y_{12}$ receptor

The $P2Y_{12}$ receptor is one of the most important targets for antiplatelet drugs. Its molecular identification was reported by Hollopeter *et al.* [26] in Nature in 2001 and it belongs to the G protein-coupled receptor family. The tissue distribution of this receptor was long thought to be restricted to platelets and subregions of the brain. Further studies later revealed its expression and functions in microglial cells, vascular smooth muscle cells, dendritic cells, macrophages, and as yet unspecified leukocytes [27]. ADP is the natural agonist of this receptor, while ATP and a wide range of its triphosphate analogues behave as antagonists.

The $P2Y_{12}$ receptor is the molecular target of the antiplatelet drugs ticlopidine, clopidogrel, and prasugrel, three thienopyridine compounds whose active metabolites are formed in the liver and covalently bind to the receptor [28,29]. It is also the target of ticagrelor (AZD6140) and cangrelor (AR-C69931MX), which are direct, reversible antagonists of the receptor [30,31]. Ticagrelor has been reported to non-competitively antagonize the $P2Y_{12}$ receptor, suggesting that its binding occurs at a site distinct from the ADP binding site [32]. Very recent structural studies have revealed how this receptor behaves when it binds agonists and antagonists or a mimetic (AZD1283) of ticagrelor. In the latter case, this allowed visualization of an adjacent binding site, close to the agonist site. However, AZD1283 was found to behave as a competitive antagonist [33–35]. In addition, these reports confirmed the role of the cysteine at position 97 as a key moiety for the action of the active metabolites of thienopyridine compounds [28,29].

The $P2Y_{12}$ receptor is responsible for completion of the platelet aggregation response triggered by ADP, which is initiated by the $P2Y_1$ receptor, for the ADP-dependent amplification of secretion, procoagulant activity, and aggregation and finally for stabilization of the platelet thrombus induced by other agents [3]. Thus, the $P2Y_{12}$ receptor is responsible for the major amplification loop represented by the release of ADP and this makes it a very attractive target for antithrombotic drugs.

Due to its central role in almost all platelet functions, the $P2Y_{12}$ receptor is importantly involved in normal primary hemostasis. $P2Y_{12}$ knockout mice display a markedly prolonged bleeding time, while patients with severe $P2Y_{12}$ deficiency may experience severe hemorrhage [31]. Similarly, animals treated with high-dose $P2Y_{12}$ inhibitors display prolongation of the bleeding time to over 30 min and may bleed to death. Hence, the issue of the bleeding risk is of major importance when $P2Y_{12}$ receptor inhibitors are used, alone or in combination with other drugs.

The $P2Y_{12}$ receptor is coupled to the inhibition of adenylyl cyclase activity through activation of a Gαi2G protein subtype [3] and subsequent PI3Kβ activation, which plays an important role in resultant amplification responses and integrin activation [36]. Another way in which $P2Y_{12}$ contributes to modulate platelet aggregation through Gαi2 involves inhibition of the cAMP-dependent protein kinase (PKA)-mediated phosphorylation of various intracellular signaling proteins, including vasodilator-stimulated phosphoprotein (VASP), an intracellular actin regulatory protein [37]. VASP does not play a key part in integrin activation but is an important marker of the $P2Y_{12}$ receptor activation state, especially to monitor the effects of antiplatelet drugs targeting $P2Y_{12}$ [38].

As the PI3K pathway is an important signaling pathway triggered by $P2Y_{12}$, one might ask whether targeting this intracellular enzyme would be safer than targeting the receptor itself. PI3K-deficient mouse models and pharmacological PI3K inhibitors have indeed been shown to display interesting antithrombotic profiles, while better preserving normal hemostasis as compared to strong inhibition of the $P2Y_{12}$ receptor. However, these are preclinical data, which do not necessarily predict the outcome in a clinical setting [36].

The thienopyridine compounds

Large-scale clinical trials have demonstrated the beneficial effects of thienopyridines in the prevention of major cardiac events after coronary artery stent insertion and in the secondary prevention of major vascular events in patients with a history of cerebrovascular, coronary, or peripheral artery disease [9,10]. The second-generation compound clopidogrel was first compared to aspirin in the CAPRIE trial in a very large population of patients with CAD, PAD, or stroke and found to be slightly more effective in reducing the combined risk of ischemic stroke, myocardial infarction, or vascular death at a dose of 75 mg day^{-1}, designed to ensure 50% inhibition of the platelet aggregation induced by ADP [39]. The success story of clopidogrel was boosted by its combination with aspirin in a series of trials, which clearly established the efficiency of this dual antiplatelet therapy, especially in the setting of PCI (see the above-mentioned reviews). The situation changed with the results of the CHARISMA study, which indicated that dual antiplatelet therapy was not significantly more effective than aspirin alone in reducing the rate of myocardial infarction, stroke, or death from cardiovascular causes in a broad population of patients at high risk for atherothrombotic events [40]. In addition to these disappointing results, clopidogrel was found to display weaknesses with regard to its pharmacokinetic properties, that is, a slow onset of the effect of the drug which, despite loading doses, requires several hours to achieve optimal inhibition of platelet functions, as likewise a slow offset due to the irreversible nature of its mode of action. Prasugrel is a third-generation thienopyridine compound having a higher efficacy and faster onset of action than clopidogrel [30]. This is due to a change in the molecule and a slightly different metabolic pathway, which result in a better rate of active metabolite generation as compared to clopidogrel. A large-scale clinical trial, TRITON-TIMI 38, including 13 609 patients planed for PCI demonstrated the overall superiority of prasugrel (60 mg loading dose, 10 mg maintenance dose) over clopidogrel (300 mg loading dose, 75 mg maintenance dose), with a 19% reduction in ischemic events and in particular a 52% decrease in stent thrombosis [41], but with a 32% increase in major bleeding, including fatal bleeding. Although not really surprising, these results had an important impact on the practises of interventional cardiologists [42].

Despite its limitations, the success story of clopidogrel proved that the $P2Y_{12}$ receptor is a very attractive target for antiplatelet drugs. Consequently, extensive research has now led to multiple compounds acting in different ways. Among these, in addition to prasugrel, two new classes of molecules have emerged as alternatives to clopidogrel, namely the oral direct antagonist ticagrelor and the intravenous short-lived compound cangrelor.

Ticagrelor

The orally active compound ticagrelor (AZD6140) is a cyclopentyltriazolopyrimidine, which is a reversible antagonist of the $P2Y_{12}$ receptor and also has an inhibitory effect on the type 1 equilibrative nucleoside transporter 1 (ENT1), thereby inhibiting adenosine reuptake. The subsequent increased concentration of extracellular adenosine has pleiotropic beneficial effects in the vasculature [43]. Ticagrelor (180 mg loading dose followed by 90 mg twice a day) results in profound inhibition of platelet aggregation. Despite its reversible mode of action, complete offset of the effect of the drug requires 3–5 days. Ticagrelor afforded improved cardiovascular outcomes, including a reduction in myocardial infarctions and vascular events, as compared to clopidogrel in the PLATO trial [44]. Moreover, in this study, it led to a notable decrease in cardiovascular and total mortality, which was not observed in the TRITON-TIMI 38 trial comparing prasugrel and clopidogrel. The main adverse events associated with ticagrelor are dyspnoea and ventricular pauses. Both these adverse events and the beneficial effect on mortality could be related to the additional 'adenosine' action of ticagrelor [43].

Several clinical trials on the use of ticagrelor in various clinical situations are currently in progress, which should help clinicians to choose between drugs in the growing family of compounds targeting $P2Y_{12}$ and to manage the different clinical settings they may encounter [8–10]. Among the numerous questions to address, one is whether all patients should benefit from dual antiplatelet therapy or whether aspirin might be dispensable, given the very potent inhibition of platelet aggregation achieved with the latest $P2Y_{12}$ antagonists. A clinical study is being conducted in the context of CAD, whereby the patients are randomized to cease aspirin or not after 1 month of standard dual antiplatelet therapy with ticagrelor and aspirin (GLOBAL LEADERS, NCT01813435).

In the CAPRIE trial, clopidogrel displayed real superiority over aspirin in patients with PAD, which might mean that targeting the $P2Y_{12}$ receptor could be better than targeting COX1 in this form of atherothrombosis (CAPRIE). The EUCLID trial (NCT01732822) is an ongoing study comparing ticagrelor and clopidogrel in patients with PAD, to see whether more profound platelet

inhibition might result in a better outcome. The study is expected to be completed by July 2016. Still in the CAPRIE trial, clopidogrel did not show any significant superiority over aspirin in stroke patients. Ticagrelor is currently being compared to aspirin in patients with acute ischemic stroke or TIAs (SOCRATES, NCT01994720), and results are expected by October 2015.

Cangrelor

Cangrelor (ARC69931MX) is an intravenously administered ATP analogue, a $P2Y_{12}$ antagonist with a very short half-life, a very rapid onset of action (3–6 min) and a very short offset (30–60 min). In the CHAMPION-PHOENIX trial comparing the standard care (aspirin plus clopidogrel) with cangrelor added to aspirin plus clopidogrel, cangrelor was clearly of benefit in the prevention of death from any cause, myocardial infarction, and stent thrombosis [45]. Thus, cangrelor has unique properties, which make it a drug for acute situations and patients waiting for surgery. It might also be helpful in patients who cannot take oral medication. Although not yet FDA approved, it has just been approved by the European EMA agency (January 2015).

GPIIb/IIIa

Glanzmann's thrombasthenia platelets fail to aggregate in response to any agonist, including ADP, TXA_2, collagen, or thrombin. This is because they lack the glycoprotein (GP) IIb/IIIa or $\alpha_{IIb}\beta_3$ integrin, the key receptor in the most important and common final pathway of platelet aggregation [4]. This platelet-specific integrin is the receptor for fibrinogen and also binds fibronectin, vitronectin, and VWF. Upon platelet activation and so-called inside-out signaling, it undergoes conformational changes, which allow it to bind soluble fibrinogen and simultaneously trigger intracellular signaling. The ligation of fibrinogen results in receptor clustering and outside-in signaling, which leads to more stable platelet aggregation and ultimately clot retraction. The cloning and sequencing of GPIIb/IIIa generated extensive research culminating in the design of a very long list of inhibitors, among which only the injectable forms are still employed, exclusively in hospital settings. Three compounds are currently in use, the monoclonal antibody abciximab, the synthetic non-peptide inhibitor tirofiban, an RGD mimetic derived from the venom of the snake Echis Carinatus, and eptifibatide, a synthetic cyclic heptapeptide patterned on a KGD motif derived from the venom of the pygmy rattlesnake Sistrurus miliarius barbouri with selectivity for α_{IIb}.

The use of these drugs is now limited to patients with a low bleeding and high thrombotic risk, for a short period of time during the acute phase immediately before and after PCI. The success of the current standard care with aspirin and drugs targeting $P2Y_{12}$, together with anticoagulation using bivalirudin or low molecular weight heparin, has decreased the need for GPIIb/IIIa antagonists. Moreover, due to their very high propensity to induce bleeding complications, these drugs are not recommended for use in stroke patients [46]. One of the undesirable side effects of $\alpha_{IIb}\beta_3$ inhibitors is thrombocytopenia. A number of mechanisms have been proposed to explain this phenomenon, including the presence of antibodies to the murine component of abciximab or antibodies to neoepitopes exposed and/or created by binding of the drugs to the receptor [4]. Another limitation is the very narrow therapeutic index, which means that more than 90% receptor occupancy is required to achieve efficacy.

Oral GPIIb/IIIa inhibitors have also been tested but with an unexpected poor outcome and serious adverse effects, both thrombotic and hemorrhagic, which are still not well understood and the clinical development was cancelled [47]. Once again, the mechanistic explanation proposed is a possible change in conformation leading to paradoxical fibrinogen binding and thrombosis along with thrombocytopenia and bleeding. Today, despite great reluctance of the drug companies to develop new $\alpha_{IIb}\beta_3$ antagonists, there is ongoing work aimed at designing new compounds, which should not induce deleterious conformational changes [48,49]. Whether such molecules will 1 day be added to the list of approved $\alpha_{IIb}\beta_3$ inhibitors is an open question.

Specific points and remaining questions

The current 'philosophy' of antiplatelet therapy, which emerged from all the clinical trials and real life experience, is to consider both the ischemic and the bleeding risk, which differ from one clinical situation to another. Basically, as already stated for aspirin, but it is valid for all drugs and all drug regimen, when the ischemic risk is high and the bleeding risk is low, strong inhibition of platelet functions is beneficial. However, the price to pay for increased efficacy is increased bleeding, which is one of the reasons for the need of new targets and new antiplatelet drugs.

Efficacy may depend on the patient's response to the drug and, during more than a decade, there was intense debate on aspirin resistance, interindividual variability of the response to clopidogrel and relevance of biological monitoring of the response to antiplatelet [50–52]. Briefly, true aspirin resistance is very rare and does not deserve any measurement. The well-documented correlation between low responsiveness to clopidogrel, mostly due to variable rates of metabolism, and recurrence of ischemic events, has led to clinical trials aimed at adapting the dose to an optimal biological response either using an automated aggregation test or the VASP phosphorylation test [52]. The negative results of these trials were disappointing and the reasons for this are still matter of debate. Anyhow, the new $P2Y_{12}$ inhibitors are stronger

and display less variability in the response, which offers new options and makes the monitoring less crucial to ensure efficacy.

Another emerging topic, and yet not fully answered question, is about the duration of dual antiplatelet therapy depending on the patient's profile and whether a bare metal or a drug-eluting stent is implanted. The recent dual antiplatelet therapy (DAPT) study [53] has shown that extending dual antiplatelet therapy beyond 1 year reduced the risk of stent thrombosis and of major adverse cardiovascular and cerebrovascular events in patients with drug-eluting stents. Again, this was accompanied by a greater risk of bleeding, although the rate of fatal bleedings was not different between the groups. Of note, at cessation of thienopyridine treatment, an elevated risk of stent thrombosis and myocardial infarction was observed during 3 months. On the other hand, several studies indicate that unnecessary prolongation of dual antiplatelet therapy with $P2Y_{12}$ inhibitors may result in increased bleeding events without real benefit in terms of efficacy [54]. This may appear contradictory but may also mean that one has to consider the patient's profile, the type of stent, and quality of stent implantation and that, at this stage, the outcome is not only related to the target but also to other determinants among which environmental factors are also to be considered.

Alternative targets for new antiplatelet drugs

Currently, under clinical investigation are compounds targeting the PAR1 receptor, the GPVI collagen receptor and the GPIb-VWF axis.

The thrombin receptor PAR1

Thrombin is the most potent platelet agonist. Human platelets express PAR1 and PAR4, which display different affinities for thrombin, PAR1 being sensitive to nanomolar concentrations of the agonist while PAR4 requires higher concentrations. The PAR receptors exhibit a unique proteolytic self-activation mechanism resulting from cleavage of their N-terminal extracellular portion and ligation of the neo N-terminus [55]. Thus, targeting the PAR1 receptor could inhibit the effects of thrombin on platelets without impairing its effects on coagulation or other functions. Two compounds have entered phase II and III clinical trials, namely atopaxar and vorapaxar. Vorapaxar, a potent oral inhibitor of PAR1, was tested in two phase III clinical studies, TRACER and TRA°P2-TIMI 50 [56,57]. The TRACER trial, which included 12 944 patients presenting ACS without ST-segment elevation, was prematurely stopped because vorapaxar increased the rate of bleeding, including serious bleeding and intracranial hemorrhage, without reducing the primary cardiovascular efficacy endpoint. One should note, however, that vorapaxar was tested against placebo in the presence of dual antiplatelet therapy. Hence, the chances of obtaining improved efficacy without increased bleeding were low. In the TRA°P2 trial, 26 449 patients with atherosclerotic vascular disease were randomly assigned to receive the thrombin antagonist vorapaxar or placebo in addition to standard therapy including aspirin, clopidogrel, or both. Vorapaxar reduced the rate of cardiovascular death, myocardial infarction, or stroke but increased the rate of moderate or severe bleeding, including intracranial bleeding. The study was conducted to its end but discontinued for patients with a history of stroke. Patients with a history of myocardial infarction derived the most benefit from PAR1 inhibition, especially if elderly patients and those with a low body mass and/or previous stroke are excluded. Vorapaxar is now FDA approved for the treatment of cardiovascular events in patients with a history of myocardial infarction or PAD but is contraindicated in subjects with previous stroke, TIAs, or active bleeding. In addition, it has to be administered together with standard antithrombotic care. This is a real limitation and stresses the urgency to dispose of results from new clinical trials in which the PAR1 antagonist should be tested alone against another drug or placebo.

GPVI

GPVI, the principal collagen receptor of platelets, belongs to the immunoglobulin-like family of receptors signaling through the FcRγ chain. Some exhaustive reviews have recently been published which deal with GPVI as a target for antiplatelet drugs in various situations [58,59]. Briefly, GPVI could be the most ideal target for antiplatelet drugs for the following reasons: it is absolutely platelet specific; its inhibition or deletion is without great impact on normal hemostasis; its pharmacological targeting with antibodies or gene targeting efficiently prevents experimental thrombosis in various models although not in all of them, especially when strong thrombin signaling is involved, which overcomes defective collagen GPVI signaling [60,61]. There are several ways to target GPVI including with antibodies, small molecules, or a dimeric GPVI-Fc fusion protein (Revacept) [62–64]. Interestingly, the fact that GPVI is apparently more strongly involved in pathological thrombus formation than in normal hemostasis makes it an attractive target to prevent ischemic stroke. Thus, experimental work in mice indicated reduced cerebral infarction and inhibition of thrombosis without increased bleeding using either an antibody such as JAQ1 [65] or the GPVI-Fc fusion protein [66]. Currently, under development are Fab fragments of monoclonal anti-GPVI antibodies and Revacept, the latter being the most advanced clinically. It was found to inhibit collagen-induced platelet activation while preserving normal hemostasis in a phase I study [67] and has now entered a phase III clinical trial where patients suffering from symptomatic carotid artery stenosis, TIAs, amaurosis fu-

gax, or stroke and presenting with microembolic signals (MES) receive either Revacept (single dose) and antiplatelet monotherapy (aspirin or clopidogrel) or monotherapy alone with the aim of reducing MES (NCT01645306). This study should end soon if it has not yet terminated when this paper appears. Concerning small molecules, the drug losartan, an AT1 receptor antagonist used to treat hypertension, displays GPVI inhibitory effects [68].

The GPIb-VWF axis

The absence of the platelet GPIb-V-IX complex results in Bernard–Soulier syndrome, a rare bleeding disorder characterized by macrothrombocytopenia [69]. Platelets interact with the damaged vessel wall through binding of the GPIb-V-IX complex to VWF immobilized on exposed collagen [70]. This mediates the initial attachment of platelets to the exposed subendothelium, which then results in firm adhesion and activation of the cells through various pathways. Circulating VWF also binds to existing platelet aggregates through the GPIb-V-IX complex, thereby participating in the recruitment of platelets and in thrombus growth. Thus, targeting the GPIb-VWF axis is thought to interfere with the early and late steps of thrombus formation, in contrast to aspirin and drugs targeting $P2Y_{12}$, which inhibit the amplification loops of platelet aggregation. As the role of this axis is most important at high shear rates, it was reasoned that it might be relevant to target the GPIb-VWF interaction in stroke models. Fab fragments of anti-GPIbα antibodies have indeed been shown to protect mice from experimental stroke without inducing excessive bleeding. [65]. Some recent studies focused on nanobodies such as the compound ALX-0081 directed against the A1 domain of VWF, or aurintricarboxylic acid (ATA), which also targets the A1 domain and inhibits the interaction between GPIbα and VWF [71,72]. In both cases, the authors concluded to the prevention of thrombosis, the restoration of vessel patency, and limited bleeding, which nevertheless remains to be confirmed in humans. A few clinical trials have been conducted using ARC1779, an aptamer with so-called VWF inhibitor activity. One study in patients undergoing endarterectomy showed a beneficial effect of this compound, while it also looked to be promising for the treatment of patients with thrombotic thrombocytopenic purpura [73–76]. Targeting the GPIb-VWF axis would therefore appear to be promising to treat ischemic stroke. However, the bleeding risk of this therapeutic strategy will need to be assessed in further clinical studies.

Other targets

Many other potential platelet targets are currently being evaluated in preclinical studies, mostly in mouse models, which require confirmation in humans, and this is of course not trivial. One main limitation is that candidate drugs have to be tested in the presence of standard antiplatelet care. Compounds targeting the purinergic receptors $P2Y_1$ or $P2X_1$, prostaglandin receptors (IP and EP1-4), or intracellular targets like PI3Kβ display interesting properties in that they prevent experimental thrombosis in various models with limited prolongation of the bleeding time as compared to clopidogrel or $\alpha_{IIb}\beta_3$ antagonists. However, the question is whether these molecules would perform better than the existing drugs. One final issue not discussed in this review is the 'dual pathway' approach, where new oral anticoagulants are added to antiplatelet drugs in patients with a history of ACS, not only during the acute phase but also during the maintenance phase of treatment [77,78].

Conclusions

A lot still remains to be done to improve the long-term efficacy in the therapy of ACS and the management of stroke and PAD. The best targets for antiplatelet drugs are probably known. Various drugs affect these targets in different ways, which has implications for their use. As the recent clinical trials have shown, the price to pay for higher efficacy is an increased bleeding risk, which has to be carefully managed. In the near future, we should find out whether new receptors or signaling molecules might efficiently replace the currently targeted $\alpha_{IIb}\beta_3$ integrin, $P2Y_{12}$ receptor, and COX1, or whether better combinations of existing drugs or a mixture of both strategies will be adequate to address the majority of the clinical manifestations of ischemic diseases.

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