Novel Strategies in Anti-Platelet Treatment for Coronary Artery Disease

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Abstract: Treatment with oral anti-platelet agents constitutes a cornerstone in the therapy of coronary artery disease. Coronary angioplasty and stent implantation improved the therapy of coronary artery disease and especially the treatment of acute myocardial infarction. Implementation of glycoprotein IIb/IIIa inhibition further advanced anti-platelet therapy as a central component in the treatment of acute coronary syndromes.

Sustained prevention of reocclusion was achieved when dual anti-platelet therapy had been introduced and reduced the risk of stent thrombosis to ~1% following elective stenting in stable coronary artery disease. However, targeting more complex lesions or performing the intervention in states of increased platelet reactivity such as in acute coronary syndromes or in diabetic patients is still associated with a higher risk of stent thrombosis. Additionally, incomplete ADP-receptor inhibition by thienopyridine treatment contributes to increased cardiovascular events and mortality after coronary intervention.

This review describes the underlying pathophysiology leading to coronary atherothrombosis and contributing to stent thrombosis as well as the pharmacological approach to prevent it by dual anti-platelet therapy. It summarizes the assessment of anti-platelet therapy by different analytical methods such as platelet aggregation, platelet function analyzers, and the platelet reactivity index. Impaired clopidogrel responsiveness and its implication for adverse cardiovascular events and stent thrombosis are discussed. Current strategies in improving the efficacy of clopidogrel treatment as well as the next generation of anti-platelet substances such as novel thienopyridines and non-thienopyridine $P2Y_{12}$-receptor blocking agents are addressed. Finally, we discuss the potential of von-Willebrand factor aptamers compared to glycoprotein IIb/IIIa inhibitors in acute coronary syndromes.

Keywords: ADP, platelet activation, $P2Y_{12}$ receptor, clopidogrel, glycoprotein IIb/IIIa.

INTRODUCTION

Platelet activation is a major component in the pathogenesis of acute coronary thrombosis and myocardial infarction. Therefore, anti-platelet therapy has become a cornerstone in the therapy of ischemic heart disease. Although adhesion molecules such as von Willebrand factor (vWF) and P-selectin initiate the first contact of the platelet to the vessel wall, the generation and release of agonists stored in platelet granules is – together with the activation of the coagulation cascade - the main mechanism of further platelet recruitment and occlusion of the blood vessel during later thrombus formation. Interventional cardiology started with sole percutaneous coronary balloon angioplasty, but this resulted in relatively rapid re-occlusion of the dilated lesion necessitating frequent reinterventions. Invention of bare metal stents made it possible to achieve higher and longer patency with less reocclusion. While patency was improved compared to pure balloon angioplasty, restenosis was a considerable concern. The availability of drug-eluting stents and the easier deliverability of currently available stents led interventional cardiology further advance and nowadays tackle more difficult and complex lesions than initially.

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STENT THROMBOSIS

While higher vessel patency as a postinterventional angiographic result had been achieved quite early in the stenting era, subacute stent thrombosis has remained as a highly dangerous complication. Stent thrombosis most commonly occurs within the first month after stent implantation, and in this interval, it is referred to as "subacute stent thrombosis." In the majority of cases, stent thrombosis is a catastrophic event, resulting in life-threatening complications. Angiographically documented stent thrombosis is associated with an incidence of death or myocardial infarction of 64%. The rate for subacute stent thrombosis varies depending on the complexity of the lesion and accompanying disease conditions such as diabetes, impaired renal function, heart failure and acute coronary syndromes (ACS). Dual anti-platelet therapy substantially reduces the incidence of early major adverse cardiac events after stent placement and is therefore recommended by guidelines for percutaneous coronary interventions [1].

In the current era of dual anti-platelet therapy, the average reported occurrence of subacute stent thrombosis is ~1%. The premature discontinuation of thienopyridine therapy is associated with a marked increase in the risk of stent thrombosis and is the leading independent predictor for stent thrombosis [2]. Late stent thrombosis following implantation

1567-2700/09

of drug-eluting stents (DES) has been reported after premature anti-platelet therapy discontinuation [3] as well as under stable anti-platelet monotherapy (e.g. actetylsalicylic acid (ASA)) [4]. A 3-year follow-up documented stent thrombosis in 1.8% of patients treated with DES. The overall incidence and the occurrence of late stent thrombosis were similar for sirolimus- and paclitaxel-eluting stents. Nearly half of all stent thromboses were encountered late, with the majority occuring after more than one year [5]. More recent data demonstrate lack of endothelial coverage of DES even at about 1 year after implantation [6], increased vasospasm and endothelial dysfunction at and distal to the DES [7, 8], as well as a high rate of thrombus-occluded DES at autopsies [9].

Consequently, current guidelines recommend dual anti-platelet therapy for a minimum of 1 month following bare-metal stent (BMS) implantation and 12 months after DES implantation if patients are not at high risk of bleeding, and emphasize that premature discontinuation of dual anti-platelet therapy should be avoided [1, 2].

PATHOPHYSIOLOGY OF THROMBUS FORMATION

Dual anti-platelet treatment is needed until the metal stent struts become adequately endothelialized to reduce the risk of stent thrombosis. Missing endothelialization results in the exposure of subendothelial matrix, which is a surface highly attractant to circulating platelets. At the site of a stented lesion, which has a thrombogenic surface similar to a vulnerable coronary plaque, platelets attach to the vessel wall and initiate thrombotic occlusion of the coronary vessel leading to myocardial ischemia and infarction, if their thrombogenic function is not blocked. Adhesion and aggregation of platelets at sites of vascular injury is mediated by vWF, a large multimeric glycoprotein present in endothelial cells, platelets and the blood plasma. vWF is critical for both physiological haemostasis and pathologic thrombosis [10, 11]. Interestingly, platelets have no measurable interaction with soluble vWF in the circulation but adhere promptly to exposed immobilized vWF. Among the constituents of the subendothelial matrix, fibrillar collagen is of major importance for vWF-mediated adhesion and aggregation of platelets. Collagen-immobilized vWF and its interaction with the platelet receptor glycoprotein (GP) Ib-V-IX establishes the initial contact between circulating platelets and the vessel wall [11]. Thereby, recruitment of circulating platelets to the site of injury and reduction of their velocity is the principal function of GP Ib-V-IX in order to enable the interaction of other platelet receptors with the thrombogenic surface. Circulating vWF associates with exposed collagen fibrils following plaque rupture, either in the course of the disease or after angioplasty, and thereby initiates platelet adhesion *via* GP Ib-V-IX through the vWF A1 domain [12]. The interaction of vWF with GP Ib-V-IX is characterized by a fast association rate that can tether platelets to the exposed subendothelium [13]. In addition to its primary function as an adhesion receptor, GP Ib-V-IX may also act as a signal transducer that activates GP IIb/IIIa and thereby supports firm platelet adhesion independently of classic activation receptors, such as G protein–coupled receptors or GP VI [12] (Fig. **1**). This novel mechanism initiates thrombus formation under high haemodynamic forces at the interface between immobilised and soluble vWF independent of precedent GP IIb/IIIa activation. This step is mandatory for platelets to participate in firm adhesion resulting in the formation of a first platelet layer which is the basis for further thrombus formation. This strictly vWF-dependent platelet activation might be of important pathophysiologic relevance in small blood vessels, stenotic arteries and acute thrombotic occlusion, therefore potentially requiring special therapeutic attention [14].

Adhesion of platelets leads to rapid platelet activation, which is mainly characterized by amplification of platelet reactivity following granula secretion. Among all the platelet agonists present at the site of the vessel lesion, adenosine-5'-diphosphate (ADP) plays a pivotal role in haemostasis and is probably the most important *in vivo* mediator of platelet activation and thrombosis. As depicted in Fig. (**1**), ADP acts mainly through two G-protein-coupled P2 receptors, $P2Y_1$ and $P2Y_{12}$ [15], which both contribute to platelet shape change, the initial step of platelet activation that precedes platelet aggregation [16]. The $G_{\alpha q}$-coupled $P2Y_1$ receptor initiates ADP-induced aggregation *via* phospholipase C (PLCβ) and mobilization of calcium. The $G_{\alpha i}$-coupled $P2Y_{12}$ receptor is involved in amplification of the aggregation response mainly *via* activation of phosphatydilinositol 3 kinase and calcium regulation [17]. The activation of platelet ADP-receptors is depicted in Fig. (**2**). $P2Y_{12}$ receptor signalling completes activation of the GP IIb/IIIa receptor (fibrinogen receptor, integrin $\alpha_{IIb}\beta_3$) and stabilizes the platelet thrombus. Due to its key role in thrombus formation, the $P2Y_{12}$ receptor has become an established antithrombotic target in cardiovascular diseases.

Platelet aggregation and thrombus formation are mediated by platelet-platelet crosslinking of fibrinogen bound to activated GP IIb/IIIa. This receptor is normally found in a resting, non-activated state on platelets which does not bind soluble fibrinogen, however, may bind to immobilized ligands under certain conditions. Platelet agonists like thrombin or ADP induce a conformational change in the extracellular domain of GP IIb/IIIa to permit fibrinogen binding and complete platelet aggregation. This change in conformation is triggered by receptor-activated intracellular signaling pathways including protein kinase C, phosphatidylinositol 3 kinase and Rap1b, that initiate a cascade of signaling events and finally regulate interaction of cytoskeletal proteins with the short intracellular domain of the GP IIb/IIIa and subsequent activation of this receptor [18, 19]. However, many aspects of these complex signaling networks still remain to be determined and may open potential new therapeutic targets in the future.

TREATMENT OF ACUTE CORONARY THROMBOSIS

GP IIb/IIIa receptor-mediated platelet aggregation plays a crucial role in arterial thrombosis. It binds fibrinogen, vWF, or other ligands, that can crosslink platelets. Anti-platelet agents blocking the binding sites on GPIIb/IIIa are efficacious in arterial thrombosis animal models and in human disease. A monoclonal antibody fragment (c7E3 Fab) and agents modelled after the arginine-glycine-aspartic acid (RGD) cell binding motif were developed for use as GP

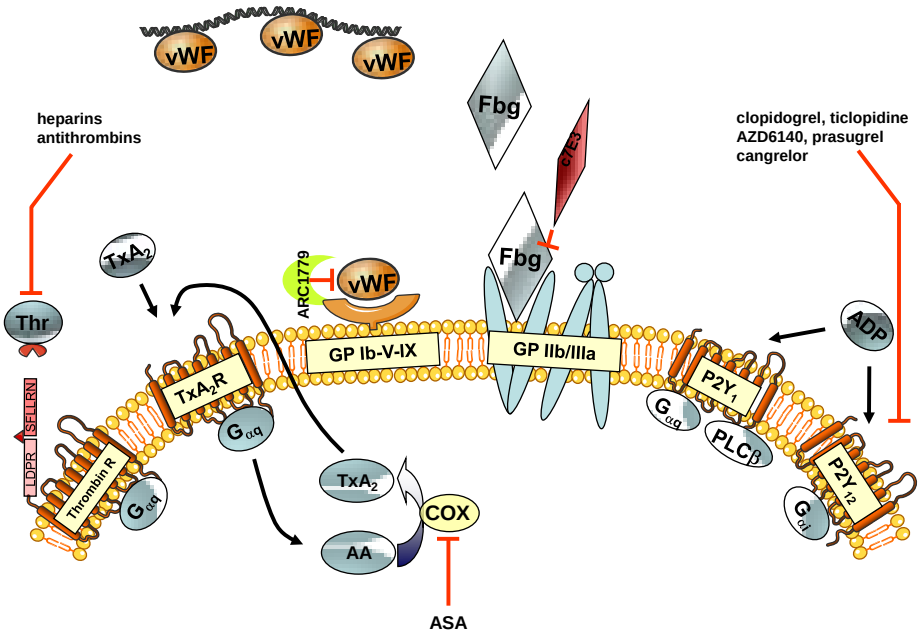

Fig. (1). Model of platelet receptor activation and its clinical pharmacological inhibition. Central elements of platelet activation and aggregation at sites of stenotic blood vessels are the binding of von Willebrand factor (vWF) to the platelet GP Ib-V-IX receptor complex and binding of fibrinogen to its platelet receptor GP IIb/IIIa. Activation of platelets can further be initiated by thrombin (Thr) or thromboxane A_2 (TxA_2) through binding to specific seven-transmembrane-spanning receptors which are linked to the intracellular platelet signalling apparatus *via* G-proteins ($G_{\alpha q}$). The important platelet agonist ADP uses three receptors for complete platelet activation, the $P2X_1$ (not depicted) receptor and the functional $P2Y_1/P2Y_{12}$ receptor signalling system which is also linked to intracellular signalling cascades *via* G-proteins ($G_{\alpha q}$ and $G_{\alpha i}$). Pharmacological inhibition of platelet activation in the clinical context is achieved mainly through specific receptor antagonists (ARC1779-vWF receptor; c7E3- antibody and small molecula antagonists-fibrinogen receptor; clopidogrel and related substances-$P2Y_{12}$ ADP receptor) or actetylsalicylic acid (ASA) that inhibits platelet granule thromboxane synthesis from arachidonic acid (AA) catalyzed by cyclooxygenase-1 (COX).

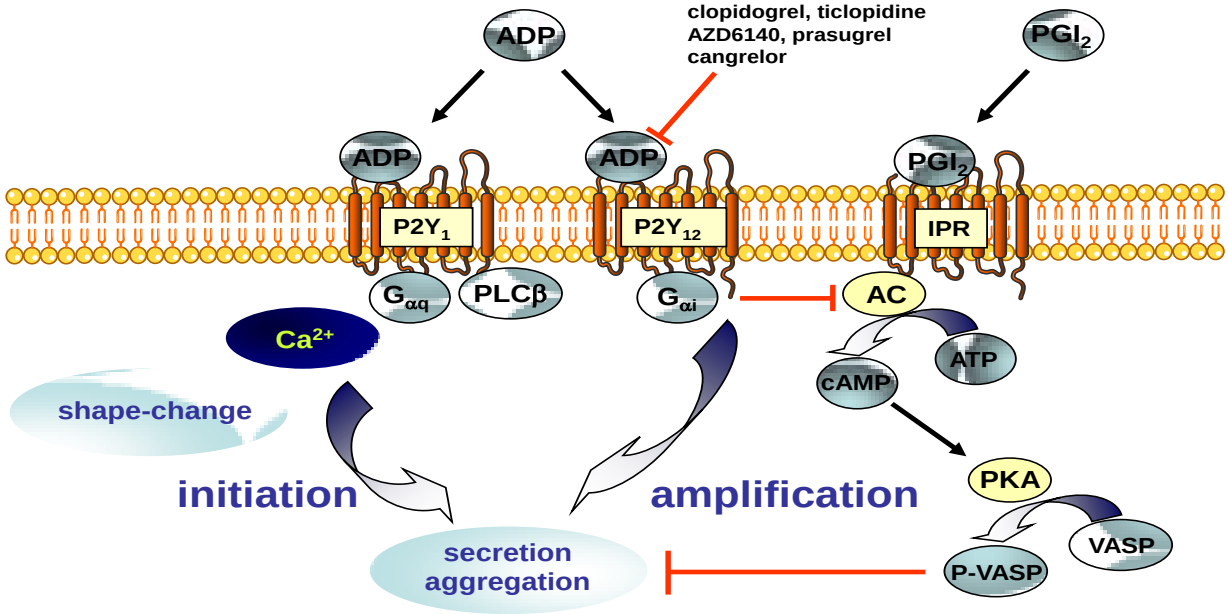

Fig. (2). Adenosine-5'-diphosphate (ADP) activates platelets through two G protein-coupled P2 receptors, $P2Y_1$ and $P2Y_{12}$. The $G_{\alpha q}$-coupled $P2Y_1$ receptor initiates ADP-induced aggregation *via* phospholipase C (PLCβ) and mobilization of calcium (Ca^{2+}), while the $G_{\alpha i}$-coupled $P2Y_{12}$ receptor mediates amplification and completion of the aggregation response. In addition, $P2Y_{12}$ is linked to the inhibition of cyclic adenosine monophosphate (cAMP) production by adenylyl cyclase (AC), which can be stimulated by prostaglandins (e.g. PGE_1 or PGI_2). cAMP-dependent protein kinase (PKA) phosphorylates the vasodilator-stimulated phosphoprotein (VASP) and inhibits platelet aggregation and secretion. The extent of ADP-induced attenuation of prostaglandin-mediated VASP phosphorylation is used in the platelet reactivity index to determine $P2Y_{12}$-specific ADP-activity. Thienopyridines and new ADP-antagonists inhibit multiple pro-aggregatory actions of ADP, mostly by preventing the $P2Y_{12}$-mediated secondary ADP-response contributing to amplification of platelet activation.

IIb/IIIa antagonists [20]. Initial experiments with c7E3 showed complete blockade of ADP-, thrombin-, or epinephrine-induced platelet aggregation as well as fibrinogen binding to platelets. It could be demonstrated that a single anatomic site is crucial to the binding of all fibrinogen molecules on platelets and that this site is on the GP IIb/IIIa complex [21]. Since binding of fibrinogen to platelet GP IIb/IIIa is a central step of platelet aggregation and thrombus formation, antagonism of GP IIb/IIIa has emerged as a rapid and reliable approach for acute antithrombotic treatment in ACS. In early studies on patients with acute MI, abciximab not only maintained patency of large coronary vessels at the site of angioplasty, but improved the recovery of microvascular perfusion and enhanced the recovery of contractile function compared to standard anticoagulation [22]. This benefit translated into reduction of 30-day rate of major adverse cardiac events [23]. Intravenous application of several GPIIb/IIIa antagonists such as the monoclonal antibody fragment abciximab as well as the small-molecule agents eptifibatide and tirofiban nowadays constitutes guideline-recommended first-line therapy in ACS with [24] and without [25, 26] ST-segment elevation undergoing early invasive therapy. However, despite the well proven efficacy of GP IIb/III antagonism, this mechanism of platelet inhibition is not unproblematic. The binding of GP IIb/IIIa ligands or of the ligand-mimetic peptide RGD causes a conformational change of GP IIb/IIIa from the non-activated to the activated state. GP IIb/IIIa inhibitors –at least in low concentrations- can have platelet activating properties resulting in fibrinogen binding to GP IIb/IIIa and consequently in platelet aggregation [27], as illustrated in Fig. (**3**). Because of their proven efficacy when applied intravenously in acute settings, oral GP IIb/IIIa inhibitors were developed for chronic treatment. However, all of the oral inhibitors showed no benefit or even increased mortality in clinical trials. Different dosing regimens during acute and chronic, intravenous and oral applications were used. The acute treatment targets for a high level of platelet inhibition while the chronic use aims for lower levels of inhibition. Low bioavailability of oral formulations led to large differences in drug levels between applications. Together with their ability to activate platelets through a GP IIb/IIIa-mediated process low trough levels with oral inhibitors may even increase platelet aggregation. This would explain the unfavourable results observed and, therefore, oral GP IIb/IIIa antagonists were not broadly used in clinical routine [28, 29].

Nevertheless, the benefit of this treatment strategy is well accepted, but major bleeding complications can occur due to the generalised inhibition of platelet aggregation by GPIIb/IIIa antagonism [27]. A more novel approach targets the interaction of platelet GP Ib and activated vWF on the vessel wall, which at sites of vascular lesions is facilitated by high shear forces. GP Ib-vWF binding does not contribute to haemostasis under low shear conditions [30] and therefore might not lead to bleeding complications in non-high-shear vascular beds such as the venous circulation or the capillaries. Several years ago, the first anti-human GPIb antibody was found to prevent platelet adhesion and thrombus formation *in vivo* in a baboon model. However, platelet-platelet interactions *in vivo* were not relevantly modified [31]. A recombinant human GPIbα chimeric protein, GPG-290, abolished cyclic flow reductions in a dog model with acute plate-

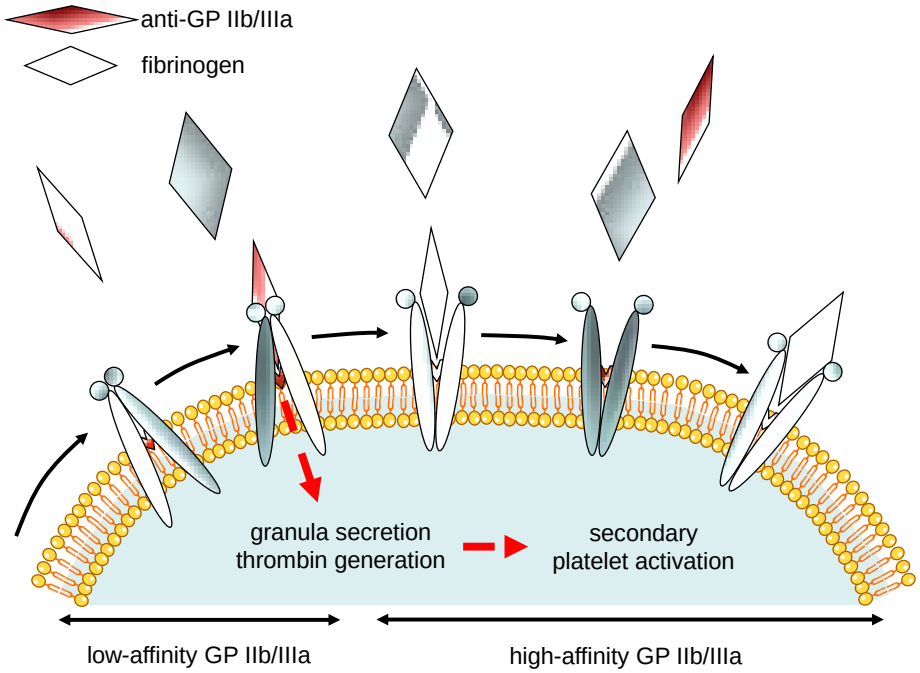

Fig. (3). Illustration of the intrinsic activity of GP IIb/IIIa antagonists. Soluble fibrinogen can not bind to low-affinity GP IIb/IIIa. Binding only occurs after an activation-triggered change in conformation of GP IIb/IIIa. While this is physiologically induced by platelet inside-out signaling following stimulation by other agonists or platelet tethering and activation of GP Ib-V-IX, GP IIb/IIIa antibodies as well as low-molecular-mass antagonists can bind to resting, low-affinity GP IIb/IIIa and induce the conformational change. While fibrinogen binding is blocked, different intrinsic activities can be exerted. Following dissociation of low-molecular-mass antagonists, GP IIb/IIIa is left in its high-affinity state.

let-mediated coronary thrombosis without prolongation of bleeding time [32, 33]. Another promising compound is the synthetically manufactured vWF-aptamer ARC1779, which displays comparable antithrombotic efficacy to the GPIIb/IIIa antagonist abciximab but does not prolong bleeding time to the same degree in a primate model of arterial thrombosis. ARC1779 was recently successfully tested for proof of mechanism and safety in humans [34].

PHARMACOLOGICAL PREVENTION OF CORONARY THROMBOSIS BY DUAL ANTI-PLATELET THERAPY

Various pharmacological possibilities to prevent platelet aggregation by oral medications are currently available, however, two approaches have been established for long-term therapeutical platelet inhibition. While ASA irreversibly inhibits cyclooxygenase (COX)-1 and thereby prevents platelet granula secretion and platelet activation by various agonists, thienopyridines selectively inhibit ADP-induced platelet activation *via* direct interaction with platelet ADP receptor signaling. Both treatments are very effective anti-platelet regimens, however, the combination of both strategies has proven even more effective in cardiovascular patients at high risk.

ADP RECEPTOR ANTAGONISM

Thienopyridines (ticlopidine, clopidogrel) inhibit multiple pro-aggregatory actions of the platelet agonist ADP by selectively and irreversibly blocking the $P2Y_{12}$ platelet ADP receptor and preventing the secondary ADP-triggered amplification of platelet activation (Fig. **1**). Compared to ticlopidine, clopidogrel has an increased pharmacological activity and less side effects. Clopidogrel requires hepatic metabolization by an cytochrome P450 isoenzyme, however, approximately 85% of the inactive prodrug clopidogrel are already hydrolyzed by circulating blood esterases to an inactive metabolite before the remaining 15% undergo hepatic metabolization to generate the active metabolite [35]. This active metabolite forms disulfide bridges with the extracellular part of the $P2Y_{12}$ receptor and inactivates the receptor in this way. Large clinical trials demonstrated the superiority of clopidogrel to aspirin for preventing ischemic events in selected patients with atherosclerotic disease [36, 37].

Low-to-intermediate risk patients undergoing elective PCI do not require GP IIb/IIIa antagonism when they are pretreated with an increased loading dose of the ADP receptor antagonist clopidogrel [38]. ACS patients with neither ST-segment elevations nor raised troponin levels were found to safely undergo PCI without accompanying infusion of abciximab, whereas troponin-positive patients profited from triple anti-platelet therapy including GP IIb/IIIa antagonism, ASA, and clopidogrel [39].

The experience that PCI in the absence of ST-segment elevations and heightened troponin levels could be performed with ASA and clopidogrel alone demonstrates the central role for inhibition of amplification of platelet activation by $P2Y_{12}$ receptor antagonism for coronary interventions.

Current guidelines from the American College of Cardiology (ACC)/ American Heart Association (AHA)/ Society for Cardiovascular Angiography and Interventions (SCAI) as well as from the European Society of Cardiology (ESC) recommend (class IB) that clopidogrel 75 mg daily should be given for at least 4 weeks after stent implantation [1, 40]. Both guidelines recommend a 300 mg loading dose of clopidogrel at least 6 hours prior to PCI [1, 40]. The ESC guidelines further recommend to initiate the loading dose even a day before the procedure if possible [40]. The daily dose of 75 mg clopidogrel had been chosen based on initial data in healthy volunteers demonstrating approximately 50% inhibition of platelet aggregation by this dosing regimen [41]. However, some patients experience thromboembolic events despite dual anti-platelet therapy, and so-called aspirin and clopidogrel "non-responsiveness" has been observed [42-46].

ASSESSMENT OF $P2Y_{12}$ INHIBITION

The method most commonly used to assess treatment efficacy of clopidogrel is conventional ADP-induced light transmission platelet aggregation, which measures the change in light absorbance through platelet-rich plasma in response to the agonist ADP. This assay needs rapid *in vitro* preparation, is labour-intensive, not well standardized and not specific for $P2Y_{12}$ receptors [47, 48]. The method itself has a high interindividual variability and only adequately assesses the platelet inhibitory effect of the therapy when pre- and post treatment samples are obtained [49].

Another routine laboratory method is electrical impedance aggregometry which can be performed in diluted whole blood in the presence of ADP. While the use of whole blood makes this assay technically less challenging, the responses are still variable and this technique has only rarely been used to investigate clopidogrel responsiveness [50]. Multiplate electrode whole blood aggregometry is a novel method for assessment of platelet inhibition by various inhibitors. Using ADP stimulation in the absence and presence of prostaglandin E_1, this technique gives rapid results from a whole blood assay, however, its specificity for $P2Y_{12}$ inhibition is not optimal compared to more specific assays [51].

Point-of-care assays such as the platelet function analyzer PFA-100 are widely used to test platelet activation under high shear stress conditions using test cartridges containing a membrane coated with type I collagen and ADP [52]. It represents an easy to use system, is automated, rapidly produces results, and only requires a small sample volume. However, its sensitivity to detect drug-induced defects of platelet function is low [53]. The PFA-100 device has failed to reliably detect thienopyridine-induced inhibition of ADP-mediated platelet activation and does not correlate with platelet aggregation [54, 55] or $P2Y_{12}$ specific assays [56]. Therefore, it is commonly accepted that the PFA-100 is not an adequate method for monitoring thienopyridines effectiveness [57].

A more recent development to monitor responses to clopidogrel treatment is the Ultegra Rapid Platelet function Assay (RPFA)-Verify Now $P2Y_{12}$. It is a modification of a point-of-care bedside test, which was initially developed to assess the anti-platelet effects of GP IIb/IIIa antagonists and is based on the measurement of agglutination of fibrinogen-coated beads by stimulated platelets in citrated whole blood.

The VerifyNow-$P2Y_{12}$ is a rapid assay that tests platelet activity over 3 min and by using a combination of ADP and prostaglandin E_1 to measure the effects of clopidogrel on the $P2Y_{12}$ receptor [58].

The probably most specific assay to determine clopidogrel non-response is the measurement of cAMP-dependent phosphorylation of the vasodilator-stimulated phosphoprotein (VASP) [59]. In addition to the amplification of platelet activation, $P2Y_{12}$ receptor stimulation also inhibits cAMP production by inhibition of adenylyl cyclase (AC) as shown in Fig. (**2**). AC is an essential enzyme in platelet inhibition by endogenous vasodilators from the group of prostaglandins. By costimulation of platelets *in vitro* with ADP and prostaglandins, this inhibitory clopidogrel effect on AC activation by prostaglandin can effectively be used to determine and quantitate the activation of $P2Y_{12}$ *via* ADP [60]. This test system is based on flow cytometry and assesses the difference in PGE_1-induced, cAMP-mediated phosphorylation of VASP in the absence and presence of ADP [61]. The remaining platelet reactivity is calculated and expressed as $P2Y_{12}$ platelet reactivity index (PRI) and is currently the only standardized $P2Y_{12}$-specific assay broadly available. If the $P2Y_{12}$ receptor is completely inhibited, the PRI is 0% because of the inability of ADP to inhibit adenylyl cyclase *via* $P2Y_{12}$, and a PRI of 100% indicates complete ineffectiveness of clopidogrel [62]. Since pre-treatment variability of this assay is very low, PRI is currently the only test selectively assessing $P2Y_{12}$-mediated signalling without requiring a pre-treatment blood sample to be diagnostic [47, 63].

IMPAIRED RESPONSIVENESS TO CLOPIDOGREL

The currently recommended daily dose of 75 mg clopidogrel is based on a phase II trial in healthy volunteers. In this study, daily doses of 100 mg or 150 mg clopidogrel resulted in marked prolongation of bleeding time compared to 75 mg, while there was no relevant additional inhibition of platelet aggregation [41]. The term clopidogrel "non-response", or initially "resistance", was introduced after clopidogrel 75 mg/day had been initiated as a standard therapy following PCI. Initially, variability of clopidogrel response was reported, when strong platelet reactivity to ADP despite a 300 mg loading dose and a 75 mg maintenance dose of clopidogrel had been found [64]. Several larger observational trials could later confirm this phenomenon: there was marked interindividual variability in clopidogrel response measured by platelet aggregation, activation of GP IIb/IIIa determined by PAC-1 antibody, which exclusively binds to the fibrinogen binding sites of activated GP IIb/IIIa, and the expression of P-selectin in response to ADP. Furthermore, the patients with the highest pre-treatment response to ADP remained the most reactive after one day [44]. Similarly, variability in response to clopidogrel has been found in patients with intermittent claudication: while a small proportion of these patients responded excessively to clopidogrel, another small group showed no response at all [65]. This phenomenon has now been widely described and its hypothetical implications have been discussed [47-49, 66, 67].

The high variability ranging from nearly complete inhibition of ADP-induced platelet activation to almost complete failure of detectable platelet inhibition raised the question, whether patients with less pronounced inhibition would be at risk for thrombotic complications, e.g. subacute stent thrombosis following PCI. Several studies demonstrated that maximum aggregation of >50% as well as a PRI >50% were associated with increased stent thrombosis and major adverse cardiovascular events [63, 68-70]. A first, retrospective analysis using $P2Y_{12}$-specific PRI among 1,684 consecutive patients undergoing PCI compared patients who had suffered a subacute stent thrombosis with stented patients free of subacute stent thrombosis. Patients with stent thrombosis had a significantly higher PRI (63%) compared to patients without stent thrombosis (39%) [63]. Another study with 100 patients who suffered subacute stent thrombosis verified that high post-treatment platelet reactivity and incomplete $P2Y_{12}$ receptor inhibition are indeed risk factors for the development of subacute stent thrombosis [71]. Afterwards, ischemic event occurrence was prospectively examined over six months and high platelet reactivity was discovered as a novel risk factor for ischemic events after PCI [70]. Especially patients with high pre-treatment reactivity at more than 2 hours after receiving the higher loading dose of 600mg had an increased risk for development of major adverse cardiac events within 30 days after elective PCI [72].

Depending on the criteria used to define "non-responsiveness", up to 30% of patients undergoing coronary stenting are clopidogrel "non-responders" [43-45, 62, 70] that might therefore have an increased risk for recurrent or subacute stent thrombosis [68, 71, 73]. The CREST-study demonstrated that patients with subacute stent thrombosis showed higher platelet reactivity determined by ADP-induced platelet aggregation, PRI, and GP IIb/IIIa activation [71]. Retrospectively, several other studies have shown that patients with stent thrombosis display impaired response to antiplatelet therapy and, therefore, incomplete $P2Y_{12}$-receptor inhibition might confer a risk factor for stent thrombosis [63, 68, 74]. A residual platelet reactivity of >50% was associated with an increased risk for stent thrombosis and adverse events [63, 69, 70]. Different study groups have recently reported that the level of platelet aggregation before coronary stenting in patients pre-treated with clopidogrel is correlated with short-, mid-, and long-term outcome after the procedure [70, 72, 75]. Clopidogrel "non-responsiveness" is also associated with increased risk of adverse cardiovascular events in patients with acute myocardial infarction [45, 76]. In patients with acute ST segment elevation myocardial infarction, who showed inadequate response to clopidogrel as determined by baseline and repetitive ADP-induced aggregation after 6 days, 40% sustained a recurrent cardiovascular event during a 6-month follow-up [45].

The pathophysiologic origin of clopidogrel non-response is still not fully elucidated and several mechanisms have been discussed (reviewed by Siller-Matula *et al.* [59]): Non-compliance and underdosing occur probably more often than expected, especially if the patient ignores the loading dose that is essential for rapid onset of the platelet inhibitory effect. However, variable absorption of clopidogrel as well as variable metabolism and clearance of the active metabolite might be involved in inadequate clinical effects. Furthermore, $P2Y_{12}$ receptor polymorphisms as well as variations of hepatic cytochrome P450-dependent metabolisation have

been discussed in the context of reduced clopidogrel effects [77, 78], however, this alone does not explain the high incidence of clopidogrel non-responders.

CURRENT STRATEGIES TO IMPROVE THE EFFICACY OF CLOPIDOGREL TREATMENT

The high variability in clopidogrel responsiveness appears to be predominantly related to inefficient metabolisation of the prodrug clopidogrel to its active metabolite [46]. *In vitro* addition of the specific, directly active $P2Y_{12}$ antagonist AR-C69931MX has been shown to overcome the observed clopidogrel non-responsiveness resulting in transient aggregation only, which is highly suggestive for complete $P2Y_{12}$ inhibition. Furthermore, a PRI of <5% was achieved in blood from patients who had an initial PRI of ~60% under chronic treatment with clopidogrel [79].

The significant inter-individual variability in pharmacological response to clopidogrel suggests a potential benefit by increasing the maintenance dose of clopidogrel, which is a strategy currently not yet generally recommended by guidelines [47, 49]. However, current guidelines include a class IIb recommendation stating that "in patients in whom subacute stent thrombosis may be catastrophic or lethal, platelet aggregation studies may be considered and the maintenance dose of clopidogrel increased from 75 mg to 150 mg/day if <50% inhibition of platelet aggregation is demonstrated" [1]. Increasing the maintenance dose of clopidogrel in patients with insufficient clopidogrel response has been shown to improve $P2Y_{12}$ inhibition demonstrated by enhanced reduction of PRI [80]. The occurrence of major adverse cardiac events following PCI is increased in patients with a PRI >50% [63, 81], while a PRI <50% has a negative predictive value of 100% [81]. In non-ST segment elevation myocardial infarction patients undergoing coronary stenting, a 600-mg loading dose of clopidogrel showed a benefit on platelet reactivity and clinical prognosis [76]. Furthermore, trials using higher loading or maintenance doses suggest that a suboptimal bioavailability of the active metabolite might contribute to clopidogrel non-responsiveness [43, 76, 80, 82]. Recently, the use of ticlopidine as an alternative therapy in the case of clopidogrel non-response has been suggested [83]. However, this approach is based on three cases observed and requires further investigation in clinical trials if more potent novel anti-platelet agents might not become available.

NEXT GENERATION OF THIENOPYRIDINES AND NON-THIENOPYRIDINE $P2Y_{12}$-RECEPTOR BLOCKING AGENTS

Since metabolic differences might contribute to the variability in response to clopidogrel [84, 85], novel substances which are metabolized more consistently and achieve more reliable platelet inhibition are needed. One such novel orally active thienopyridine prodrug with potent and long-lasting anti-platelet effects is prasugrel. The anti-platelet potency of prasugrel is at least 10 times higher than that of clopidogrel and first promising results have been shown in patients undergoing elective PCI [86, 87]. In healthy subjects, prasugrel (60 mg loading dose) inhibited platelet aggregation more effectively and more rapidly than clopidogrel (300 mg loading dose). Its effects start already 15 min after oral application. Furthermore, the response to prasugrel was more consistent compared to clopidogrel and the lower inhibition of platelet aggregation by clopidogrel was associated with lower plasma concentrations of its active metabolite [84]. In stable aspirin-treated patients with coronary artery disease no non-responders (defined as inhibition of platelet aggregation by less than 20%) to prasugrel were detetcted, while the rate for clopidogrel-treated patients was 45%. Furthermore, prasugrel given with a 40-60 mg loading dose and maintained at 10-15 mg daily achieved greater inhibition of platelet aggregation in this population [86]. Recently, the TRITON-TIMI 38-study compared prasugrel to clopidogrel in moderate to high risk patients with acute coronary syndrome and PCI. Treatment with prasugrel significantly reduced ischemic events including stent thrombosis, however, it was also associated with significantly increased bleeding risk including fatal bleedings and major bleeding during coronary artery bypass grafting [88].

Cangrelor is an intravenously administered, reversible agent, which has a rapid onset of activity and inhibits $P2Y_{12}$ reversibly. It inhibits platelet aggregation with rapid onset and offset and does not require metabolism for therapeutic activity [67, 89]. Given as an adjunct to reduced thrombolytic therapy it has a similar safety profile than full-dose thrombolysis in patients with acute myocardial infarction and achieves similar coronary patency [90].

A new class of $P2Y_{12}$ receptor antagonist are the cyclopentyltriazolopyrimidines. The reversible $P2Y_{12}$ receptor antagonist AZD6140 is the first of these new compounds of anti-platelet agents. It is given orally and does not require metabolic activation [89]. Similar to thienopyridines, AZD6140 blocks the platelet $P2Y_{12}$ receptor to inhibit the prothrombotic effects of ADP. Unlike thienopyridines, which are irreversible antagonists, AZD6140 binds reversibly to the $P2Y_{12}$ receptor at a distinct, noncompetitive site [91]. For AZD6140, maximum inhibition of platelet aggregation is observed already after 2-4 hours, whereas clopidogrel only minimally inhibits platelet aggregation at this time. AZD6140 produces approximately 90-95% inhibition of platelet aggregation 4 hours after dosing during chronic treatment, while clopidogrel reaches only approximately 60% inhibition [91]. AZD6140 showed no difference in major bleeding in patients with non ST elevation ACS [92], but reduced platelet aggregation more rapidly and effectively compared with clopidogrel. AZD6140 induced greater inhibition of platelet aggregation than a standard regimen of clopidogrel and further suppressed platelet aggregation in patients pretreated with clopidogrel [93]. The efficacy and safety of AZD6140 is currently assessed in the phase 3 trial PLATO in patients with acute coronary syndromes.

CONCLUSIONS

Thienopyridines, especially clopidogrel, have a highly significant effect in clinical trials with regard to reduction of stent thrombosis and functional inhibition of ADP-induced platelet activation in cardiovascular patients. Nevertheless, there is a high incidence of impaired clopidogrel responsiveness associated with adverse cardiovascular events following PCI. Individual testing and adjusted anti-platelet therapy might be recommendable under certain circumstances, e.g.

high-risk interventions in the last patent or a dominant vessel. Further prospective studies are needed to determine the risk reduction by individually adjusted anti-platelet therapy. Newly developed $P2Y_{12}$ antagonists in combination with inhibitors of other platelet receptors that are currently under development or in clinical trials might be able to provide safer and more efficient anti-platelet therapy in cardiovascular medicine in the near future.

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Received: March 12, 2008 Revised: July 14, 2008 Accepted: July 14, 2008