Thienopyridines in cardiovascular disease: Focus on clopidogrel resistance

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Summary
Platelets play an important role in atherothrombotic disease, as well as in the pathogenesis of atherosclerosis and in complications. Antiplatelet therapy with clopidogrel represents at present an important treatment of coronary artery disease (CAD), especially in and after acute coronary syndromes (ACS), and after coronary interventions when stents are used. Clopidogrel is a potent and specific inhibitor of platelet ADP receptor (P2Y12 receptor) with high anti thrombotic activity. Emerging data suggest that a significant percentage of individuals treated with clopidogrel do not receive the expected therapeutic benefit because of a decreased responsiveness of their platelets, which is caused by several extrinsic and/or intrinsic mechanisms. As long as clopidogrel is the “gold standard” in combination with aspirin in the treatment of patients undergoing percutaneous coronary intervention and stent implantation, the overall challenge is to develop a fast “point-of-care” assay to detect clopidogrel resistance early and to enable alternative anti-thrombotic strategies in non-responders or low-responders. This test should be easily performed (bedside) and reproducible, with a standardized definition of response, which is known to correlate with clinical outcomes. Unfortunately, such a test does not exist at present. As an alternative, new ADP receptor antagonists with better bioavailability and improved pharmacokinetics, e.g. intestinal reabsorption as an active drug or 1:1 conversion into an active metabolite thus reducing individual variations, are in development and have already found their way into clinical use in phase-3 trials. Prasugrel is one of the incoming new drugs with high expectations, but other agents might follow in the near future.

Keywords
ADP receptors, platelet pharmacology, clopidogrel resistance

Introduction
Antiplatelet drugs have an established place in the prevention of vascular events in a variety of clinical conditions, such as myocardial infarction (MI), stroke and cardiovascular death (1). Activation and aggregation of platelets plays a central role in the propagation of intracoronary thrombi after spontaneous atherosclerotic plaque disruption that results in myocardial ischemia or infarction (1). At the site of vascular lesions, platelet adhesion to the exposed matrix is the initial step in thrombus formation. Platelets adhere to exposed subendothelium through interactions with a variety of platelet surface receptors. Among those is the glycoprotein (GP) Ib-IX (2), which is the main receptor for the subendothelial protein ligand von Willebrand factor (vWF). Upon contact with collagen, platelets become activated via GP VI (3) and generate and release secondary agonists such as thromboxane A2 and adenosine diphosphate (ADP), which in combination with thrombin generated by the coagulation cascade results in stimulation and recruitment of additional platelets (1). This is followed by clustering and activation of the glycoprotein GP Ib/IIa receptor (4). Subsequently, platelets spread and form a surface for the recruitment of additional platelets via fibrinogen bridges between GP Ib/IIa (αIIbβ3) receptors. The local concentration of tissue factor initiates this extrinsic clotting cascade and leads to coagulation, the generation of more thrombin, and the propagation of a fibrin clot (Fig. 1).

Clopidogrel sensitive receptor: P2Y12

The P2Y12 receptor subtype of ADP receptors has been found in human platelets (5), microglia (6), retina (7) and in bovine chromaffine cells (8) as well. This receptor is coupled to G protein, inhibits adenylate cyclase, activates IP3-kinase, and regulates ion channels (9) (Fig. 2). The P2Y12 receptor plays a crucial role in...
thrombus formation and stabilization. The P2Y<sub>12</sub> receptor augments the activating signal for platelet aggregation and promotes platelet release reaction. Stimulation of P2Y<sub>12</sub> is essential for ADP-mediated complete activation of GP IIb/IIIa and GP Ia/IIa, and further stabilization of platelet aggregates (5). P2Y<sub>12</sub> receptor signalling plays a crucial role in thrombin-induced ERK-2 activation in human platelets (10). Moreover, the P2Y<sub>12</sub> receptors play a significant role in the development of platelet micro aggregates in patients with diabetes (11). The P2Y<sub>12</sub> receptor is down-regulated in patients with systemic lupus erythematosus (SLE), and a decreased concentration of the P2Y<sub>12</sub> receptor might represent a protective response in SLE against thrombotic complications (12). Recent reports have shown that P2Y<sub>12</sub> inhibition has anti-inflammatory effects as well (13). Ticlopidine, clopidogrel and prasugrel (CS-747, LY640315) are specific antagonists of the P2Y<sub>12</sub> receptor (14, 15). Further antagonists are ATP and its triphosphate analogues (16), reactive blue 2 (RB2) (17), six 2-alkylthio-substituted ATP analogues, including the adenosine-aspartate conjugate 2-hexylthio-AdoOC(O)Asp2, and five AR-C compounds (AR-C67085, AR-C69931, AR-C78511, AR-C69581, AR-C70300) (18, 19), which are in development.

**Thienopyridines: pharmacology and metabolism**

Ticlopidine, clopidogrel and prasugrel belong to the thienopyridine family of ADP-receptor antagonists, which act via irrevers-
ible inhibition of the platelet P2Y$_{12}$ receptor. In the clopidogrel and prasugrel molecules, the presence of a methoxycarbonyl group provides an increased pharmacological activity and a better safety and tolerability profile compared with ticlopidine (20) (Fig. 3). Besides strong antitrombotic activity, thienopyridines have other pharmacological effects, including stimulation of nitric oxide production (21), inhibition of erythrocyte aggregation (22) and reduction of circulating fibrinogen levels (23). In particular, clopidogrel also affects platelet aggregation by collagen and thrombin (24). Several pro-inflammatory events, including the release of CD40 ligand (24) and the expression of P-selectin (25) are reduced by clopidogrel treatment.

The thienopyridines in current clinical use are pro-drugs, requiring hepatic bioactivation by the cytochrome P450 isoform 3A4 in order to generate the active metabolite, a transient intermediate, which covalently modifies and inactivates the receptor in a highly specific and irreversible manner (26). At least 20 metabolites of ticlopidine have been identified. It has been proposed that UR-4501 is the molecule responsible for the in-vivo activities of ticlopidine (27). The active metabolite of clopidogrel is a reactive thiol derivative (bearing 7S, 3Z and 4S or 4R configuration) out of a family of eight stereoisomers (28). The bio-transformation of prasugrel involves rapid de-esterification (30 minutes) to R-95913 followed by formation of an active metabolite (R-138727) and its four stereo isomers, respectively (29).

The active metabolites from thienopyridines form disulfide bridges with the extracellular cysteine residues (Cys17 and Cys270) on the P2Y$_{12}$ receptor and thereby inactivate the receptor (30).

Ticlopidine doses of 250, 375, and 500 mg per day inhibit platelet aggregation by 20–50%, 30–60%, and 50–70%, respectively. Doses higher than 500 mg per day do not produce a significant further increase in the extent of inhibition. A clopidogrel dose of 75 mg per day inhibits platelet aggregation by 40–60% at steady state, which occurs within three to seven days (31). In contrast, a single oral dose of prasugrel (30 and 75 mg) produces a >50% inhibition of platelet aggregation with rapid onset (1 hour [h]) and long duration (>48 h) of action (32).

Prasugrel, clopidogrel and ticlopidine inhibit ADP-induced platelet aggregation in a dose-dependent manner with an ED$_{50}$ (a dose that reduced the response by 50%) of 1.2 mg/kg, 16 mg/kg and >300 mg/kg, respectively (32).

Reabsorption of ticlopidine is rapid. About 80% of a single dose is reabsorbed, which is further increased when the medication is taken after a meal (33). In case of clopidogrel, only 50% is reabsorbed and bioavailability is not affected by food.

Ticlopidine has been shown to be effective in peripheral artery disease, unstable angina, and cerebrovascular disease (14). However, the incidence of neutropenia and the gastrointestinal side effects associated with ticlopidine have led to the development of a second-generation thienopyridine, clopidogrel, which offers a superior safety and tolerability profile compared with ticlopidine. Clopidogrel is a widely used antiplatelet agent and is approved for the prevention of atherothrombotic events in patients with the clinical manifestation of atherosclerosis, including recent ischemic stroke, recent MI and peripheral artery disease (14).

Figure 3: Chemical structures of thienopyridines: A) ticlopidine, B) clopidogrel, C) prasugrel.
ary revascularization (38), ischemic events (39), and diabetes mellitus (40), respectively.

The CURE study (41) demonstrated that patients with unstable angina or non-ST segment elevation MI (NSTEMI) exhibited a 20% relative risk reduction if they were randomized to clopidogrel plus aspirin versus placebo plus aspirin. In an analysis, which examined the onset and duration of the therapeutic effect, the benefit of the clopidogrel loading dose was observed early as well as 24 h after randomisation and significantly reduced MI, stroke or severe recurrent ischemia (p=0.003) (42).

The PCI-CURE substudy showed that this benefit was also existent in patients undergoing percutaneous coronary intervention (PCI). The relatively slow onset of action of clopidogrel requests administration of a loading dose (300 mg) prior to interventional procedures (43). Overall, the results from PCI-CURE showed clear evidence of early and long-term benefit of clopidogrel.

The CREDO trial confirmed the findings of PCI-CURE: The maximum benefit of clopidogrel administered together with aspirin required a loading dose given at least 6 h prior to the PCI. This study also demonstrated a significant 27% reduction in death, MI and stroke after one-year administration of clopidogrel plus aspirin following PCI as compared to the use of combined antiplatelet therapy for only one month (44).

The CLARITY-TIMI 28 study was performed in patients with ST-segment-elevation MI who received a standard thrombolytic therapy and were randomized at presentation to clopidogrel, initiated with a 300 mg loading dose, followed by 75 mg/d, or placebo for an average of four days (until coronary angiography). The addition of clopidogrel to standard fibrinolytic therapy (patients included also received aspirin and heparin or low-molecular-weight heparin as adjunct therapy) resulted in a 36% relative risk reduction of a combined end point (occluded infarct-related artery or death or MI by time of angiography) (45).

The PCI-CLARITY trial was a pre-specified substudy, which investigated whether clopidogrel pre-treatment (hours to days) before PCI was superior to clopidogrel initiated at time of PCI. The trial demonstrated that pre-treatment with clopidogrel significantly reduced the incidence of cardiovascular death, MI, or stroke before and after PCI without a significant increase in major or minor bleedings (46).

The CHARISMA trial was designed to evaluate the efficacy and safety of clopidogrel plus aspirin versus placebo plus aspirin in patients with established coronary, cerebral, or peripheral artery disease, or in patients with multiple risk factors for atherothrombosis who had not yet experienced an ischemic event. The study resulted in no long-term benefit of the combined antiplatelet therapy in stable patients but exhibited an increased bleeding rate in patients receiving aspirin and clopidogrel (47).

Prasugrel

The JUMBO-TIMI 26 study compared clopidogrel with prasugrel in patients undergoing elective or urgent PCI. In this phase-2 study, which was designed to assess safety when therapy was administered at the time of PCI, both, prasugrel and clopidogrel resulted in low bleeding rates (15).

Early-phase data from the TRITON TIMI 38 study (clopidogrel vs. prasugrel in STEMI or NSTEMI patients) showed that prasugrel produces a more consistent platelet inhibition compared with clopidogrel. All patients on prasugrel responded to the drug, while 22 to 43% of clopidogrel-treated patients did not (data presented at the Cardiovascular Research Foundation’s 17th Annual Transcatheter Cardiovascular Therapeutics scientific symposium, TCT, Washington, D.C., USA, 2005).

Recently, it could be shown that prasugrel achieves a greater inhibition of platelet aggregation and a lower rate of non-responders compared with clopidogrel in aspirin-treated patients with stable coronary artery disease (48).

Clopidogrel resistance

Definition of clopidogrel resistance

The term “resistance” to a drug should be used when a drug is unable to hit its pharmacological target. The extent of platelet aggregation in vitro to ADP has been used to define clopidogrel resistance in the vast majority of published studies. Clopidogrel resistance is thereby defined as an absolute difference between baseline aggregation and post treatment aggregation (▲aggregation [%]) of 10% or less, when 5 µM ADP is used as stimulus of platelet aggregation. Because ▲aggregation [%] = baseline aggregation [%] – post treatment aggregation [%], a negative ▲aggregation would indicate post-stent platelet reactivity greater than baseline, and a positive ▲aggregation would indicate platelet inhibition (49).

Definition of high post-treatment platelet reactivity

Gurbel et al. defined high post treatment platelet reactivity as platelet aggregation in the >75th percentile range in response to 5 and 20 µM ADP in patients pre-treated with a 300-mg loading dose. These authors suggested that post-treatment platelet reactivity is a better estimate of thrombotic risk rather than clopidogrel responsiveness (50).

Clinical evidence for clopidogrel resistance

Clopidogrel resistance occurs in up to 40% of patients undergoing PCI and stent implantation. In severe cases, non-responders to clopidogrel tend to develop acute or subacute stent thrombosis with a potentially fatal outcome. Accordingly, early detection of clopidogrel resistance is of high importance as long as clopidogrel is the most frequently used thienopyridine in cardiovascular disease.

Jaremo et al. investigated individual variations of platelet inhibition after clopidogrel-loading doses in patients with stable angina. They were able to demonstrate a considerable individual heterogeneity in the platelet response to clopidogrel. Some individuals only had weak responses whereas others exhibited strong platelet inhibition (51).

Measurements of platelet aggregation and of platelet activation markers (GP IIb/IIIa and P-selectin detection by specific antibodies) disclosed clopidogrel resistance in 31% of the patients on day 5 after initiation of therapy and still 15% on day 30. The maximum inhibitory response to a 300-mg loading dose of clopidogrel followed by 75 mg/d occurred within 24 h, and the platelet inhibition at day 30 was predicted by the response at day 5 (49).

Müller et al. searched for clopidogrel non-responders among 105 patients with coronary artery disease undergoing elective
PCI. They found that 5–11% of the patients were non-responders and 9–26% were low-responders. Among the group of non-responders there were two incidents of subacute stent thrombosis after PCI (52).

A prospective study of PCI patients with NSTEMI showed that up to 25% of the patients were to some extent resistant to clopidogrel (53). When the patients were stratified into quartiles based on resistance to ADP-induced platelet aggregation, the most resistant patients had a 40% adverse event rate during a six-month follow-up period.

In another study, platelet reactivity and activation at baseline and at 2 h, 24 h, 5 days and 30 days after coronary stenting were measured in 94 consecutive patients. Patients were treated with aspirin (325 mg) and clopidogrel (300 mg loading does/75 mg per day). A significant percentage of patients receiving standard antiplatelet combination therapy exhibited high post-treatment platelet reactivity and activation, which persisted for 30 days after the procedure (54).

Angiolillo et al. compared platelet function profiles in patients undergoing coronary stenting. Patients (32%) were either pre-treated with clopidogrel (2x75 mg per day at least 48 h before intervention) or received a 300 mg loading dose at intervention (68%). Platelet aggregation as well as P-selectin and PAI-1 expression were significantly lower in clopidogrel pre-treated patients at baseline (p<0.001) and at 4 h (p<0.01), while there was no difference between groups 24 h after intervention (55).

In another study, platelet function was evaluated before and after clopidogrel therapy in 50 candidates scheduled for PCI. The failure of clopidogrel to inhibit platelets (30% non-responders) was neither related to clinical pre-treatment variables, including atorvastatin therapy, nor to major adverse coronary events (56).

Recent studies have shown that pre-treatment platelet activity did not predict clopidogrel responsiveness. Thus it appears that post-treatment platelet reactivity is a better predictor of thrombotic risk than responsiveness to clopidogrel as indicated above (57).

Gurbel et al. determined the effect of clopidogrel in different doses on the incidence of non-responsiveness and high post-treatment platelet aggregation in 190 patients undergoing coronary stenting. Patients were randomly treated with either a 300-mg or a 600-mg clopidogrel loading dose. Non-responsiveness was lower after the 600 mg dose compared with the 300-mg dose (8% vs. 28% and 8% vs. 32% with 5 and 20 µM ADP, respectively, p< 0.001). However, it could be shown that some patients with low post-treatment platelet aggregation are clopidogrel non-responsive, whereas some responders will continue to have high post-treatment aggregation. Again it could be shown that post-treatment platelet reactivity is a better estimate of thrombotic risk rather than clopidogrel responsiveness (59).

Gurbel et al. identified patients with subacute stent thrombosis treated at two tertiary care centres over a 1.5-year period. Sixty percent of the patients with subacute stent thrombosis had high post-treatment platelet reactivity (58).

In another study, 44% of patients undergoing coronary stenting had an inadequate response (low-responders) to a standard 300 mg clopidogrel loading dose. Compared to normal responders, low responders had a higher activation of the GP IIb/IIIa receptor before intervention, which remained increased up to 24 h thereafter (13).

Wenaweser et al. postulated that resistance to aspirin but not to clopidogrel appears to be associated with stent thrombosis: Patients with a history of stent thrombosis showed an impaired response to antiplatelet therapy with aspirin compared with controls and volunteers. However, additional treatment with clopidogrel was not able to overcome these differences in platelet aggregation, thus indicating that low responsiveness to aspirin and to clopidogrel might be linked in some patients (59).

Cuisset et al. reported that ADP stimulation of platelet aggregation could determine low responders as individuals with high platelet reactivity (>70%) after conventional dual antiplatelet therapy. They prospectively studied the platelet response to both, clopidogrel and aspirin in 106 consecutive patients with NSTEMI acute coronary syndrome undergoing PCI with stenting. A single post-treatment blood sample was obtained just before PCI and analyzed by platelet aggregometry using both ADP and arachidonic acid as agonists. Low platelet response to aspirin was significantly correlated with low response to clopidogrel, but contributed less to cardiovascular events (60).

Another report also demonstrated that aspirin-resistant patients as a group exhibited a reduced inhibitory response to clopidogrel in parallel (61). The relatively high incidence of CK-MB elevation after PCI in these patients suggests that they might be at high risk for thrombotic complications following coronary intervention.

### Mechanism of clopidogrel resistance

Several mechanisms of clopidogrel resistance have been discussed (Table 1). Non-compliance with clopidogrel therapy is probably an underestimated cause of “failure” to inhibit platelet activation but difficult to measure. Gencheva et al. described a substantial discrepancy (up to 20%) between the frequencies of total subject discontinuation for any reason and the sum of study drug-induced adverse events, voluntary withdrawal and loss to follow-up in patients participating in clinical trial and receiving antiplatelet therapy (62).

Another important mechanism responsible for clopidogrel resistance is inappropriate dosing of the substance. The recommended maintenance dose for clopidogrel is 75 mg per day, but without a loading-dose it takes up to five days until maximal platelet inhibition is obtained. Accordingly, when a more rapid inhibition of platelet function is required as in the pre-treatment to a planned PCI, a loading dose of 300 mg is recommended at least 6–24 h before the intervention (63). More recently it has been re-

### Table 1: Possible mechanisms of clopidogrel resistance.

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<td><strong>Extrinsic mechanisms:</strong></td>
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<td><strong>Platelet interactions with other receptors or molecules:</strong></td>
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<td>Increased platelet activity with collagen and ADP</td>
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ported that a 600-mg loading dose of clopidogrel increases the rate of platelet inhibition up to 80% already after 2 h (50, 64, 65). A 600-mg loading dose of clopidogrel given to patients already on a permanent maintenance dose of 75 mg/d is also able to further suppress platelet aggregation (64). Single doses of clopidogrel higher than 600 mg are not associated with an additional significant suppression of platelet function, most obviously due to limited clopidogrel reabsorption (66).

Other potential extrinsic mechanisms include a variable absorption of the prodrug or a variable clearance of the active metabolite. Taubert et al. investigated pharmacokinetics of clopidogrel after administration of a 600-mg loading dose. The extent of inhibition of ADP-induced aggregation varied from 33% to 78% in healthy individuals 6 h after initiation of therapy. This indicates that the variability in response to clopidogrel is predominantly caused by individual differences in clopidogrel reabsorption (67).

Other factors such as ADP receptor reactivity or differences in bioactivation of clopidogrel do not seem to play a major role (67). In summary, this wide individual variability makes clopidogrel, despite its well-known benefits, unpredictable for efficacy in the individual patients.

Recently, a drug-to-drug interaction between clopidogrel and atorvastatin has been described (68). The authors speculated that this might occur at the level of the CYP3A4 cytochrome, which generates the receptor by interacting with the generation of the active metabolite of clopidogrel. In contrast, Matezyk et al. were not able to confirm this finding (53). Also a retrospective analysis of clinical trials has shown no evidence for a significant interaction between clopidogrel and statins (69). Moreover, the antiplatelet effect of a 600-mg loading dose of clopidogrel was not influenced in patients receiving atorvastatin and simvastatin for at least four weeks prior to coronary stenting (70). Similarly, it has been shown that neither atorvastatin nor pravastatin significantly influenced clopidogrel-induced inhibition of platelet activation (71). Recently, no difference in the inhibition of ADP-induced platelet aggregation was seen in 33 patients using a lipidophilic statin (atorvastatin) versus 33 patients on a hydrophilic statin (pravastatin). The antiplatelet activity of clopidogrel also remained unchanged after a three weeks discontinuation of the respective statins (72).

Recently, polymorphisms of the P2Y12 receptor have been described and made responsible for a reduced activity of clopidogrel: ADP-induced platelet aggregation was associated with a haplotype of the P2Y12 receptor gene. Carriers of the H2 allele may have less protection of clopidogrel (73). Angiolillo et al. were able to demonstrate that the IVS10+12G>A polymorphism of the CYP3A4 gene modulates platelet activation in patients treated with clopidogrel and might therefore contribute to the variability in clopidogrel response (74). Based on the fact that there are large inter-individual variations in the CYP3A4 (75), it has been suggested that this “basal” variations in clopidogrel bioactivation might be an important factor to understand the clinical variability in antiplatelet responses to clopidogrel (72, 74).

Increased activation of the GP IIb/IIIa receptor could also be a possible reason for clopidogrel non-responsiveness. Compared to normal responders, low responders had a higher activation of the GP IIb/IIIa receptor before intervention, which remained increased up to 24 h after clopidogrel front-loading (13).

**Platelet function tests for detection of clopidogrel resistance**

Platelet assays have been studied in cardiovascular diseases to predict clinical outcomes and to monitor platelet function inhibitors as aspirin, clopidogrel or platelet aggregation inhibitors as GP IIb/IIIa-antagonists. The most frequently used technologies are assays of platelet function, i.e. aggregate formation (photometric assay according to Born, PFA-100, impedance aggregometry), measurement of expression of activation markers at the platelet surface, such as P-selectin, GP IIb/IIIa, or other platelet adhesion molecules (FACS). The alternatives are platelet reactivity tests: These test determine a platelet count ratio, for example before (EDTA) and after standardized stimulation (Ultera-RPFA, ICHOR). This will allow calculating a fraction of platelets that become activated and clot or adhere to artificial surfaces (PADAs) as a percentage of total platelet count.

In practical use all of these platelet tests have considerable limitations. Regarding the detection of “resistance”, the comparison of two different methods (PFA-100 and optical aggregometry) yielded different results even in the same patients, i.e. platelets, which were “resistant” according to one assay were not resistant in the other and vice versa and were considered “semi-responders” (76). In the absence of appropriate controls, i.e. platelet assays prior to ingestion of antiplatelet drugs, results may even disqualify patients (76). Thus, while measurement of platelet function will help to determine platelet reactivity to standard stimuli and to detect non- or low-responders *in vitro*, the data should be interpreted with caution, because so far it is not clear whether, and if so, for what extent these data correlate with the clinical outcome.

There are also some technical issues with the analysis of platelet function in platelet-rich plasma *in vitro*. The measurements have to be done rapidly after blood withdrawal because of the increase in pH with outgoing CO2 that increases platelet reactivity. The assays are dependent on the type of aggregant and anti-coagulant used. For example, citrate will reduce the Ca++ levels in the sample close to zero, while antithrombins, such as hirudin, will maintain physiological Ca++-levels, allowing for more physiological activation of the platelet GP IIb/IIIa receptor. However, the most potent natural platelet activator, i.e. thrombin, cannot work. It should also be noted that the frequently used quantification of acetyl-salicylic acid (ASA) sensitivity in terms of inhibition of ADP-induced aggregation *in vitro* is an artefact (in non-ASA pre-treated patients). ASA *in vivo*, i.e. in the presence of physiological Ca++-concentrations, will not affect ADP-induced platelet responses because there is no ADP-induced thromboxane formation (77). This issue might be particularly relevant to ADP-antagonists, because the possible explanation for a variable response might differ between patients on aspirin treatment, i.e. absent thromboxane formation, and those who are not. These limitations have prompted the European Heart Association not to recommend the measurement of platelet function as a parameter for ASA “resistance”.

The probably most specific assay to determine clopidogrel “resistance” (and resistance to other thienopyridines) is the measurement of PKA-dependent VASP-phosphorylation. This method quantifies the extent of Gq-mediated inhibition of adenylate cyclase in platelets after previous increase in adenylyl cyclase...
clase activity by a standard dose of PGE$_1$, PGE$_2$, as a chemically stable compound replaces the natural, but labile ligand PGI$_2$. This assay allows to detect and to quantify the unique property of thienopyridines to synergize with this agonist – in contrast to aspirin, which rather has the opposite effect. In addition, in contrast to all other in-vitro assays, it also considers the possible influence of the endothelium on platelet function by addition of a PGI$_1$ analogue. Unfortunately, the assay is costly and not easy to perform and, therefore, not a laboratory standard procedure.

**New antiplatelet compounds**

Currently most interest is focussed on inhibitors of purinergic receptors at the platelet surface and here on antagonists of the P2Y$_{12}$ receptor. Several compounds have entered clinical trials. This includes parenteral, reversible antagonists, such as cangrelor (AR-C69931 MX), which is highly selective and of interest for short-term use (78). AZD6140 is an orally active reversible antagonist of the P2Y$_{12}$ receptor. The antiplatelet activity starts at less than 1 h after administration and the biological half-life is about 12 h. According to one phase-II trial (DISPERSE), the compound is more potent than clopidogrel and more predictable in the clinical efficacy with side effects similar to clopidogrel (79).

The clearly most promising compound at the time is prasugrel, an orally active thienopyridine, which is converted into the active metabolite (R-138727) in a 1:1 ratio (29). Thus, there is no metabolic waste as with clopidogrel. According to two phase II trials in healthy volunteers and one Phase “I” trial in patients with coronary heart disease, the compound is considerably more potent than clopidogrel and was found to have a much higher responder rate than clopidogrel at a comparable risk of bleeding.

**Clinical implications of resistance to clopidogrel**

Recent data suggest that acute and subacute stent thrombosis is the clinical consequence of non-use of clopidogrel or clopidogrel resistance and the sufficient inhibition of platelet aggregation with clopidogrel has a pivotal importance for the prevention of stent thrombosis (80). Accordingly, the detection of clopidogrel resistance might have a central role in the prevention of acute and subacute stent thrombosis, which usually occurs before re-endothelialization has been completed. There are several predictors of stent thrombosis including stent implantation in primary PCI of acute MI, the use of drug-eluting stents and angiographic variables like multiple stent placement, incomplete stent expansion, poor ejection fraction, small stent diameter, and residual dissection (80, 81). In acute stent thrombosis, mortality rate (22% and more) and the incidence of non-fatal MI (up to 67%) are high (81).

The discrepancy between the relatively high rate of clopidogrel resistance (up to 40%) and the corresponding quite low overall incidence of acute and subacute stent thrombosis (0.6–2%) remains unclear. Obviously, the in vitro platelet function tests currently used do not reflect real in vivo conditions.

Recent findings have demonstrated an increase of late stent thrombosis and also of cardiac and non-cardiac mortality especially after the use of drug-eluting stents (82). The causes are not well defined although it is believed that failing complete re-endothelialization might also be an important contributor for this life-threatening event. In many cases the reduction of combined antiplatelet therapy 6–12 months after initiation of combined platelet therapy has been made responsible for this late complication. However, late stent thrombosis does not typically occur few days after stopping clopidogrel but can occur also weeks and months later, thus indicating the importance of other contributing factors for in-stent thrombus formation. These are not fully understood at present but may be linked to pathologic mechanisms in the vessel wall. Although such pathologic causes are difficult to identify and might consist of variable mechanisms, late stent thrombosis is the common consequence. Accordingly, the prolongation of combined antiplatelet therapy in patients receiving drug-eluting stents (up to 1 year or longer) seems an attractive strategy to prevent late stent thrombosis.

From the clinical point of view detection of resistance or hypo-responsiveness to clopidogrel should in theory end up in a change in therapy e.g. increase of dosage or addition of other compounds with platelet inhibitory capacity. At present, no data from randomized prospective studies are available, which have been designed to answer this question. This can be explained by the fact that none of the in vitro platelet function tests as described was able to reflect conditions in vivo, which in turn would allow an adaptation of therapy. The development of new assays and the clinical investigation of the most promising platelet function tests already on the market therefore have highest priority.

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