Platelet function variability and non-genetic causes

Ioannis Tentzeris1*; Jolanta Siller-Matula2*; Serdar Farhan1; Rudolf Jarai1; Johann Wojta2; Kurt Huber1

13th Medical Department with Cardiology and Emergency Medicine, Wilhelminen Hospital, Vienna, Austria; 2Department of Cardiology, Medical University of Vienna, Vienna, Austria

Summary
Dual antiplatelet therapy (DAPT) has been established for the treatment of coronary artery disease, especially in and after acute coronary syndromes, and after coronary interventions. 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 intrinsic mechanisms. The clinical consequence of clopidogrel non-responsiveness is severe cardiovascular complications. Besides genetic variability in response to antiplatelet therapy, non-genetic causes such as drug interactions (proton-pump inhibitors, statins, calcium-channel blockers, coumarine derivates, antibiotics, antimycotics) and co-morbidities (diabetes mellitus, renal failure, obesity) are responsible for this phenomenon. Large clinical trials with standardised laboratory methods and hard clinical endpoints are needed to identify these interactions with clopidogrel and predictors for its non-responsiveness.

Keywords
ADP receptors, antiplatelet agents, platelet pharmacology

Introduction
Dual antiplatelet therapy (DAPT) has been established for the treatment and secondary prevention of non-ST-elevation acute coronary syndrome, ST-elevation myocardial infarction and in stable patients after percutaneous coronary intervention (PCI) and stent implantation and is recommended of the current practice guidelines (1–3). An important problem with DAPT is the wide inter-individual variation in its antiplatelet effect (4–6). It has been demonstrated that especially clopidogrel does not exert an antiplatelet effect in over 20% of patients undergoing PCI (7). The clinical consequence of an insufficient platelet inhibition by clopidogrel or aspirin are cardiovascular complications (8–12) such as acute or subacute stent thrombosis (13–16), which can cause death and acute myocardial infarction (17).

Underlying mechanisms of an inadequate platelet response to clopidogrel are non-compliance, variable absorption or metabolism, concomitant drug interaction (proton-pump inhibitors, calcium-channel blockers, coumarine derivates, antibiotics and antimycotics; see Table 1), co-morbidities (diabetes mellitus, chronic heart or renal failure, obesity), smoking, higher age, gender and gene polymorphisms (18–20). This paper reviews non-genetic causes of this phenomenon.

Methods
We performed a literature research from PubMed for publications (prospective clinical controlled trials, reviews, meta-analyses) in English until March 2011. The keywords which have been used were “clopidogrel resistance”, “clopidogrel non-responsiveness”, “clopidogrel and drug interaction” and “platelet reactivity”.

Clopidogrel non-responsiveness
Clopidogrel non-responsiveness has been defined as the failure to inhibit platelets activity and has been evaluated with different ex vivo tests (18, 21, 22). Although there is increasing evidence that monitoring the antiplatelet effect of clopidogrel can identify patients at high risk for ischaemic events, routine monitoring has not been established yet, and standardised platelet function assays are lacking (23, 24).
Drug interactions

Proton-pump inhibitors

Clopidogrel is activated by the CYP2C19 which also metabolises proton pump inhibitors (PPIs) (25–27). PPIs are frequently administered in combination with DAPT to reduce the risk for gastrointestinal bleeding. As many PPIs inhibit CYP2C19, it has been hypothesised that PPIs might interfere with the antiplatelet effect of clopidogrel and therefore reduce its clinical benefit (28). Indeed, trials examining the effect of PPIs on pharmacodynamics of clopidogrel demonstrated that omeprazole reduces the antiplatelet effect of clopidogrel (29–33). In contrast, such a negative effect of concomitant PPI use has not been shown for pantoprazole, lanso- prazole or esomeprazole, respectively (27, 31, 32, 34). Retrospective data analyses from large registries suggested that the concomitant treatment with clopidogrel and PPIs increases the rate of major cardiac adverse events (35–37). In contrast, other studies (38–40), as well as post-hoc analyses of the randomised CREDO (41) and TRITON-TIMI 38 (42) trials showed no such correlation. In the CREDO trial and in the study of Charlot et al. the use of PPIs was associated with worse clinical outcome in both, the clopidogrel arm as well as the placebo arm (41, 43) thus suggesting that not the PPI use and its potential impact on clopidogrel efficacy itself but other factors e.g. patient-related parameters associated with the prescription of PPIs might be the responsible agents of clinical deterioration. In the study of Saraffot et al. (38), concomitant treatment with a PPI in patients receiving DAPT after coronary stenting is not an independent predictor of stent thrombosis but patients with PPI had higher mortality, probably due to confounding as patients on PPIs had a higher risk profile at baseline. The worse patient’s outcome in registry studies with large patient numbers might be explained by inclusion bias based on the fact that the sicker patients receive more frequently gastric protection with PPIs.

### Table 1: Drug-drug interactions in clopidogrel treatment.

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<thead>
<tr>
<th>PPIs:</th>
<th>Influence on pharmacodynamics</th>
<th>Influence on outcome</th>
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<tbody>
<tr>
<td>Omeprazole</td>
<td>Interaction shown in randomised controlled trials and in cohort studies</td>
<td>Inconsistent effects shown in a randomised controlled trial and in cohort studies</td>
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<tr>
<td>Pantoprazole</td>
<td>Lack of interaction shown in randomised controlled trials and in cohort studies</td>
<td>Inconsistent effects shown in cohort studies</td>
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<td>Esomeprazole</td>
<td>Lack of interaction shown in cohort studies</td>
<td>Inconsistent effects shown in cohort studies</td>
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<td>Lansoprazole</td>
<td>Inconsistent effects shown in randomised controlled trials and in cohort studies</td>
<td>Inconsistent effects shown in cohort studies</td>
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<td>Rabeprazole</td>
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<td>Inconsistent effects shown in cohort studies</td>
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<tr>
<th>CCBs:</th>
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<th>Influence on outcome</th>
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<tr>
<td>Dihydropyridines</td>
<td>Interaction shown in cohort studies</td>
<td>Inconsistent effects shown in cohort studies</td>
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<tr>
<td>Non-dihydropyridines</td>
<td>Inconsistent effects shown in cohort studies</td>
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<tr>
<th>Anticoagulants:</th>
<th>Influence on pharmacodynamics</th>
<th>Influence on outcome</th>
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<tr>
<td>Phenprocoumon</td>
<td>Interaction shown in cohort studies</td>
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<th>Antimycotics:</th>
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<th>Influence on outcome</th>
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<th>Influence on outcome</th>
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<tr>
<td>Rifampicin</td>
<td>Interaction shown in a randomised controlled trial</td>
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<tr>
<th>Statins</th>
<th>Influence on pharmacodynamics</th>
<th>Influence on outcome</th>
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<tr>
<td>Lipophilic:</td>
<td>Inconsistent effects shown in randomised controlled trials and in cohort studies</td>
<td>Inconsistent effects shown in retrospective analyses of data</td>
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<td>Atorvastatin</td>
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<td>Hydrophilic:</td>
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<td>Lack of interaction shown in cohort studies</td>
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<td>Rosuvastatin</td>
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CCB, calcium-channel blocker; PPI, proton pump inhibitor.
contrast a meta-analysis of Siller-Matula et al. demonstrated that concomitant PPI and DAPT use might be associated with an increased risk of cardiovascular events but does not influence the risk of death (44). A meta-analysis of Hulot et al. demonstrated that patients on PPIs and clopidogrel had an increased MACE (major adverse cardiovascular events) rate and mortality. The authors mentioned that this finding was observed only for high-risk patients (45). Recently, the prospective randomised COGENT trial (46), demonstrated that omeprazole reduces gastrointestinal events in patients on clopidogrel. Patients on omeprazole and clopidogrel had similar rate of cardiovascular death, non-fatal myocardial infarction, revascularisation or stroke with patients on clopidogrel alone.

The pharmacodynamic data clearly show an interaction between clopidogrel and omeprazole but not between clopidogrel and pantoprazole (34, 47). This led to warnings concerning concomitant use of clopidogrel with omeprazole/esomeprazole from the United States Food and Drug Administration (FDA) and The European Medicines Agency (EMEA) but not with pantoprazole.

There is only one trial which demonstrates a possible interaction between PPIs and aspirin. In this trial PPIs enhance the antiplatelet effect of enteric-coated aspirin (48). The authors suggested that PPI can influence the absorption of aspirin though the gastrointestinal tract.

What is of interest, co-administration of pantoprazole enhanced the antiplatelet effect of enteric-coated aspirin in patients

Figure 1: Metabolism of clopidogrel and possible drug-drug interactions.
with acute coronary syndrome undergoing PCI (48). This interaction might be due to enteric-coating, which alters the pharmacokinetics of drugs.

Statins

Clopidogrel is also activated through CYP isoenzyme 3A4 (Fig. 1), and it has been hypothesised that drugs that are substrates or inhibit this isoenzyme can interfere with the conversion of clopidogrel in his active metabolite and reduce its antiplatelet effect. Indeed, the study of Lau et al. have shown that atorvastatin but not pravastatin reduces the antiplatelet effect of clopidogrel (49) in patients after coronary stent implantation, and this effect was dose-dependent. These findings were also confirmed for lipophilic statins (50). However, larger studies have shown no interaction between statins and high loading dose (51–53) or low loading dose clopidogrel (54–61).

Calcium-channel blockers

Another class of cardiovascular drugs which is metabolised by the CYP P450 enzyme are calcium-channel blockers (CCBs; Fig. 1) (62). Forbes et al. demonstrated that the treatment with nifedipine has no effect on platelet inhibition of clopidogrel in patients with coronary artery disease or peripheral occlusive artery disease (63). Prospective cohort studies in patients undergoing stent implantation have shown that concomitant treatment with CCB results in a reduced effect of clopidogrel when CCB were administered concomitantly (64–66). In one study (64), which investigates the interaction of clopidogrel and CCBs and clinical outcome, the rate of the endpoint (cardiovascular death, non-fatal myocardial infarction, stent thrombosis or revascularisation) was significantly high in patients on CCBs. Two recent studies, which investigated the impact of CCBs on the antiplatelet effect of clopidogrel in patients after myocardial infarction (67) or coronary stenting (68), demonstrated that the clinical efficacy of clopidogrel is not reduced by concomitant treatment with CCBs.

Other drugs

There is only one study (4), which demonstrated that non-responders to clopidogrel were more often treated with angiotensin-converting enzyme (ACE)-inhibitors but there is lack of studies, that evaluate this interaction. Zahno et al. demonstrated that ketoconazole and clarithromycin can impair clopidogrel biotransformation and its antiplatelet activity (69). This effect for ketoconazole has been confirmed in healthy volunteers (70). Sulfonylureas have been associated with decreased platelet inhibition by clopidogrel after elective coronary stent implantation (71). One study demonstrated association of clopidogrel resistance and use of benzodiazepines and selective serotonin reuptake inhibitors (72).

Interestingly, phenprocoumon significantly attenuated the antiplatelet effects of clopidogrel in patients under DAPT. However, the impact of this interaction on the risk of thrombotic and bleeding events after PCI requires further investigations (73).

What is very interesting, the antibiotics rifampicin increased the inhibitory effect of clopidogrel on platelet aggregation by potentiation of clopidogrel active metabolite production (74).

No negative effects on the antiplatelet effect of clopidogrel were shown for the following drugs: donepezil (75), theophylline (76), digoxin (77), erythromycin or troleandomycin (49).

Co-morbidities

Angiolillo at al. compared the platelet function in diabetic and non-diabetic patients on DAPT (78), and demonstrated that diabetics have increased platelet reactivity compared with non-diabetics on DAPT. This is also confirm from Ang et al. (79) Lepantalo et al. (80) and Hochholzer et al. (81). In another study the insulin-dependent diabetics had greater platelet aggregation than non-insulin-dependent diabetics (82). In addition, high platelet reactivity in diabetics is associated with a three-fold increased cardiovascular event rate than diabetics without high platelet reactivity (83). Diabetes mellitus has been also been associated with aspirin non-responsiveness (84–86).

Patients with chronic renal failure have a more decreased platelet responsiveness to clopidogrel than patients with normal renal function, which could not be improved by an increase in the clopidogrel dosage (87).

Some studies have shown the association of age and body mass index to clopidogrel resistance (72, 81, 88–95). In addition, patients with unstable angina have significantly lower inhibition of platelet aggregation (93, 96) and an infectious complication during the course of an acute coronary syndrome or elevated C-reactive protein levels in stable patients lead to more pronounced platelet aggregation and is associated to aspirin non-responsiveness (95, 97, 98). Postula et al. demonstrated that a total cholesterol/high-density lipoprotein (HDL) cholesterol level ratio above 2.99 is also significantly related to aspirin non-response (99), and another study has demonstrated that the concomitant medication with omega-3 ethyl esters potentiates the antiplatelet effect of clopidogrel (100). Two studies have shown that smoking can positively influence the inhibitory effect of clopidogrel on platelets (101, 102).

Novel drugs that may overcome clopidogrel low responsiveness

According to the well-known clinical limitations of clopidogrel, new antiplatelet drugs have been established or are currently under

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development. These agents include i) blockers of the P2Y12 receptor such as prasugrel, ticagrelor, cangrelor, elinogrel and BX 667; ii) antagonists on the protease activated thrombin receptor-1 such as SCH 530348, SCH 205831, SCH 602539 and E5555; iii) terutroban, an inhibitor of the thromboxane receptor; and inhibitors of von Willebrand factor -dependent platelet aggregation such as ARC1779, ALX-0081, and AJW200, respectively (103, 104). The pharmacologic and clinical profiles of the new platelet antagonists prasugrel and ticagrelor indicate that they provide more consistent, more rapid and more potent platelet inhibition than clopido- 
grel (105, 106). The SWAP study demonstrated that for patients receiving maintenance clopidogrel therapy after an acute coronary syndrome, switching from clopidogrel to prasugrel was associated with further reduction in platelet function (107). The PLATELET substudy of the PLATO trial demonstrated that ticagrelor achieves greater antiplatelet effect than clopidogrel in patients with acute coronary syndrome, both in the first hours of treatment and during maintenance therapy (108).

Conclusion

A definition of non-responsiveness to antiplatelet therapy varies between studies. Clear definitions, laboratory tests and cut off values that can identify patients with reduced response to clopido- 
grel, who are at high risk of experiencing cardiovascular events have to be defined.

There are many controversies regarding the interaction of clopidogrel with other drugs. Therefore, prospective randomised trials with hard clinical endpoints should be performed to identify drugs that reduce the antiplatelet effect of clopidogrel. Some studies indicate that such agents might be significant in “poor metabolisers” (19).

Some co-morbidities, such as diabetes mellitus, renal failure and obesity are identified as predictors of the clopidogrel effective- 
ess. These patients may benefit if they are treated with newer drugs or higher dose (109–111). A benefit for diabetics has been demonstrated for prasugrel and ticagrelor over clopidogrel (112–114). In addition, patients with acute coronary syndrome, which have greater platelet reactivity than stable patients (21, 96), may benefit more from newer agents. These new agents may also overcome the problem of genetic polymorphism, which affect the metabolism of clopidogrel and reduces its biotransformation (19).

Conflict of interest

The author Kurt Huber received honoraria for lectures from Astra Zeneca, Eli Lilly / Daichi Sankyo and Sanofi-Aventis. None of the other authors have conflicts of interest to report.

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