The present state of aspirin and clopidogrel resistance

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Summary
Antiplatelet therapy has demonstrated significant clinical benefit in the treatment of acute coronary syndrome. However, as with any treatment strategy it has been unable to prevent all cardiovascular events. This is far from surprising when considering the complexity of arterial thrombosis and more specifically platelet physiology. This lack of treatment success has provoked the introduction of various diagnostic tests and testing platforms with the intent of guiding and optimizing clinical treatment. Such tests have resulted in the generation of clinical data that suggest suboptimal response to antiplatelet agents such as aspirin and clopidogrel. In the case of both aspirin and clopidogrel, this suboptimal response has been termed resistance. Drug resistance would imply a lack of pharmacological response that has not been specifically investigated in many of the clinical studies performed to date. Rather, the term resistance has been used to describe various facets of platelet activation and aggregation relative to the testing method. Many of these measured parameters are not addressed in the therapeutic intent of the antiplatelet drug in question.

Indications for aspirin and clopidogrel

Atherothrombosis of the coronary, cerebrovascular and peripheral arterial circulation is the world’s leading cause of morbidity and mortality (20). Platelets play a fundamental role in the pathophysiology of atherosclerosis and have become a target for therapy in both primary and secondary prevention of atherothrombotic disease. Aspirin is at the forefront of antiplatelet treatment and based on numerous studies, once daily low dose aspirin is recommended in clinical conditions where antiplatelet therapy demonstrates a favorable benefit to risk ratio (28).

A direct result of coronary heart disease, acute coronary syndrome (ACS) manifests itself in the form of unstable angina and acute myocardial infarction with or without ST segment elevation. The initial treatment may involve a conservative approach with medication, or when faced with aggressive presentation of the disease the treatment may take an invasive turn involving percutaneous coronary intervention (PCI) to restore blood flow to the occluded vessel. Often this involves the placement of a bare metal or drug-eluting stent. Clopidogrel in addition to aspirin has proven especially effective in this venue for the prevention of both restenosis and stent thrombosis in the treatment of acute coronary syndrome (5, 6, 52).

Numerous large scale clinical studies support the safety and efficacy of single or dual antiplatelet treatment in specific clinical settings. In addition to these studies a number of guidelines have been written to promote educated decisions when considering antiplatelet therapy in daily practice. Table 1 provides an overview of indications for aspirin and clopidogrel use based on suggestions from the American Heart Association (AHA), the American College of Cardiology (ACC), and the European Society of Cardiology (ESC) (12, 28). This is not a definitive list though
Aside from differing details there is general agreement in the use of aspirin and clopidogrel. Additional antiplatelet drugs may be indicated in specific clinical settings and it is always important to consider the benefit to risk ratio when administering aspirin and clopidogrel. In order to avoid unnecessary side effects, aspirin should be administered in the lowest dose that has demonstrated efficacy in the specific setting.

Platelet activation

Thrombus formation involves the activation of platelets by vascular injury or in the case of cardiovascular disease, plaque rupture. Platelets adhere to the injury site through the process of adhesion, involving a series of glycoprotein (GP) receptors on the platelet surface and their corresponding adhesive proteins including:

- GP VI – collagen,
- GP complex Ib-V-IX – von Willebrand factor,
- integrin αIIbβ₃ – fibrinogen.

Upon adhesion subsequent shape change occurs with accompanying thromboxane (TxA₂) production and platelet granule release, the contents of which include adenosine diphosphate (ADP). TxA₂ and ADP are soluble agonists that further activate the adhered platelets in addition to activating local free circulating platelets, a form of recruitment. Localized thrombin is capable of activating platelets through a third agonist pathway. Thrombin activation results in a cross linked platelet plug as fibrinogen strands bind to glycoprotein αIIbβ₃ receptors. Upon activation the αIIbβ₃ receptors physically alter their conformation producing the high affinity fibrinogen binding site GPIIb/IIIa on the platelet membrane. Thus, secondary platelet activation occurs by means of three principal agonist pathways; thromboxane (TxA₂), adenosine diphosphate (ADP), and thrombin, independent of or in combination with each other (35).

Modos of action

Aspirin

For over 100 years acetylsalicylic acid has been available for use as an anti-inflammatory and antipyretic drug, first synthesized by Hoffman in 1898 and marketed under the trade name of aspirin in 1899. Aspirin’s effectiveness as an antiplatelet agent began in the 1960’s when it was noted that aspirin prolonged the bleeding time (50). The possibility of monitoring aspirin’s antiplatelet effects in-vitro became a reality with the introduction of the Born platelet aggregometer in 1962 (7). Since that time the benefits of aspirin in preventing cardiovascular events have been well studied. Aspirin reduces the rate of myocardial infarction, stroke and cardiac death by 25% in high risk individuals (1). Despite this significant advancement, individuals continue to suffer vascular events while on aspirin therapy (24).

The means by which aspirin achieves its cardiovascular benefit is attributed to its ability to inhibit thromboxane production.

<table>
<thead>
<tr>
<th>clinical indication</th>
<th>drug</th>
<th>comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>primary prevention high risk</td>
<td>aspirin</td>
<td></td>
</tr>
<tr>
<td>• diabetes mellitus</td>
<td>aspirin</td>
<td></td>
</tr>
<tr>
<td>• hypertension</td>
<td>aspirin</td>
<td></td>
</tr>
<tr>
<td>peripheral vascular disease</td>
<td>aspirin or clopidogrel*</td>
<td>*as an alternative to aspirin</td>
</tr>
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<td>atrial fibrillation</td>
<td>aspirin</td>
<td>intermediate risk or</td>
</tr>
<tr>
<td></td>
<td></td>
<td>high risk not on warfarin</td>
</tr>
<tr>
<td>valve</td>
<td>aspirin or clopidogrel*</td>
<td>*as an alternative to aspirin</td>
</tr>
<tr>
<td>• surgery</td>
<td>aspirin or clopidogrel*</td>
<td>*as an alternative to aspirin</td>
</tr>
<tr>
<td>• disease</td>
<td>aspirin or clopidogrel*</td>
<td>*as an alternative to aspirin</td>
</tr>
<tr>
<td>ischaemic heart disease</td>
<td>aspirin or clopidogrel*</td>
<td>*as an alternative to aspirin</td>
</tr>
<tr>
<td>• acute coronary syndrome</td>
<td>aspirin or clopidogrel*</td>
<td>*as an alternative to aspirin</td>
</tr>
<tr>
<td>• no ST-segment elevation</td>
<td>aspirin or clopidogrel*</td>
<td>*as an alternative to aspirin</td>
</tr>
<tr>
<td>– with PCI</td>
<td>aspirin or clopidogrel*</td>
<td>*as an alternative to aspirin</td>
</tr>
<tr>
<td>– without PCI</td>
<td>aspirin or clopidogrel*</td>
<td>*as an alternative to aspirin</td>
</tr>
<tr>
<td>• ST-segment elevation in acute myocardial infarction</td>
<td>aspirin or clopidogrel*</td>
<td>*as an alternative to aspirin</td>
</tr>
<tr>
<td>previous MI</td>
<td>aspirin or clopidogrel*</td>
<td>*as an alternative to aspirin</td>
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<tr>
<td>elective PCI</td>
<td>aspirin or clopidogrel*</td>
<td>*as an alternative to aspirin</td>
</tr>
<tr>
<td>• resulting in stent placement</td>
<td>aspirin or clopidogrel*</td>
<td>*as an alternative to aspirin</td>
</tr>
<tr>
<td>post coronary bypass</td>
<td>aspirin</td>
<td></td>
</tr>
<tr>
<td>acute ischaemic stroke/TIA</td>
<td>aspirin</td>
<td></td>
</tr>
<tr>
<td>previous stroke/TIA</td>
<td>aspirin or clopidogrel*</td>
<td>*as an alternative to aspirin</td>
</tr>
</tbody>
</table>

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Tab. 1

Indications for use of aspirin and clopidogrel; adapted with permission from the ESC Expert Consensus Document on the Use of Antiplatelet Agents (13)
Aspirin acetylates serine residue 529 (Ser 529) in the polypeptide chain of the prostaglandin H-synthase (PGH-synthase), thus inhibiting the production of PGH2 which is the direct precursor of thromboxane (29, 34). Because platelets are anucleate they do not readily regenerate protein. The effect of aspirin on COX-1 is irreversible and lasts for the lifetime of the platelet thereby relying on the generation of new platelets to recover cyclooxygenase activity at a rate of approximately 10 percent per day in normal healthy individuals (2, 23). Low dose aspirin is sufficient to suppress greater than 95 percent of thromboxane production by COX-1 and such suppression is capable of inhibiting platelet aggregation (32). However, aspirin affected platelets may still aggregate in the presence of potent platelet agonists such as collagen and thrombin (26).

COX-2 has been identified in human atherosclerotic plaques and in newly formed platelets (41, 49). COX-2 is largely responsible for prostacyclin production which counteracts the effects of thromboxane by preventing platelet aggregation and stimulating vasodilation (2). Higher doses of aspirin are capable of inhibiting COX-2 production of prostacyclin in endothelial cells while converting the PGH-synthase-2 enzyme into a lipoxygenase. 15(R) hydroxy-eicosatetraenoic acid (HETE) is produced and invokes the anti-inflammatory effect associated with high dose aspirin (21). It is noteworthy that selective COX-2 inhibition sufficient to suppress prostacyclin production can promote arterial thrombosis. However, COX-2 present in endothelial cells maintains the ability to regain prostacyclin production only hours after exposure to aspirin due to the ability of nucleated cells to resynthesize enzymes (18).

**Clopidogrel**

Clopidogrel is a member of the thienopyridine class of drugs that target the purinergic receptors on the platelet surface. More specifically, they irreversibly bind to the adenine diphosphate receptor P2Y12 thus inhibiting platelet aggregation by diminishing signal transduction and subsequent activation of the GPIIb/IIIa receptors as well as diminishing the amplification of platelet activation through the dense granule release of ADP (9) (Fig. 2). Clopidogrel is a prodrug which must be metabolized in the liver to its active metabolites. Metabolism occurs through CYP3A4, one of the cytochrome P450 enzymes responsible for drug metabolism (9). Clopidogrel must go through two oxidation steps in order to produce an active metabolite capable of irreversibly binding to the platelet ADP receptor P2Y12.

The P2Y12 and P2Y1 are two G-protein coupled receptors that are expressed on platelet membranes. Their combined action is necessary for a full activation and aggregation response to stimulation by ADP (39, 40).

Only a small amount of the prodrug is metabolized into an active metabolite capable of binding to its target with the majority of clopidogrel undergoing hydrolysis to an inactive carboxylic acid derivative (9). Like aspirin, clopidogrel is administered in a daily maintenance dose and displays an additive antiplatelet effect over time. Because it elicits an irreversible effect for the lifetime of the platelet, it also recovers according to the degree of platelet turnover (30).

### Monitoring

Currently, many tests are available commercially and several are in development to measure the effect of aspirin and clopidogrel on platelets (Tab. 2). Multiplate®, PFA-100®, Plateletworks®, Ultegra® Verify Now, and Platelet Mapping® are assays that measure platelet aggregation or platelet agglutination resulting from ex-vivo stimulation of the platelet using a variety of platelet agonists. These tests are performed on whole blood samples and designed for point-of-care testing to provide rapid results as compared with traditional light transmittance aggregometry still considered the reference standard. The Impact and Impact-R systems mimick sheer flow and measure adhesion of platelets to endogenous adhesive proteins in the whole blood sample. Though all of the aforementioned platforms incorporate the same fundamental concept of platelet-platelet interaction, the technologies differ in respect to the aspects of platelet activation
incorporated in the individual testing method.

The AspirinWorks® and VASP tests approach drug response from a biochemical perspective. An immunoassay, the AspirinWorks test measures 11-dehydrothromboxane B₂, a urinary metabolite of systemic thromboxane production not specific to platelet generation of thromboxane. This metabolite is diminished when aspirin is effective in inhibiting the COX-1 pathway but is not eliminated completely when COX-2 induced or renal production of thromboxane is present (31, 36, 48). By means of flow cytometry, the VASP test measures the degree of phosphorylation of a vasodilator stimulated protein directly linked with the activity of the P2Y₁₂ receptor on the platelet surface and is affected by the presence of clopidogrel (4).

In addition, there exist a number of non-commercial tests available for use in the research setting that detect numerous platelet receptors and constituents exposed or released by the platelet upon activation. Many are flow cytometric assays and several are microplate based. Noteworthy of mention is a test for serum thromboxane B₂. It represents the optimal method for determining the direct ability of platelet COX-1 to produce thromboxane in the presence of aspirin. The clinical significance of this test has not been established in relation to adverse outcomes. The incidence of aspirin resistance is reportedly low as a result of COX-1 specific tests lending to the confusion over the appropriate definition of resistance (47). As a result of the varying technologies, limited correlation has been noted between many of the testing platforms available which raises the additional issue of which test is optimal for identifying resistance (38).

Identifying the risk

Though routine testing for aspirin or clopidogrel resistance is not recommended at this time due to a lack of consensus on:

- a definition,
- the exact cause(s) and
- the appropriate treatment course.

There is mounting evidence for the ability of both biochemical and functional tests to identify individuals at substantial risk for an adverse event while on antiplatelet therapy.

One metaanalysis of 15 studies and a second metaanalysis of 20 studies demonstrated a near four-fold increased risk of developing a cardiovascular event in individuals who were identified as resistant by various tests (19, 44). In two studies addressing aspirin treatment including the largest outcome study to date (n = 3261), highest levels of 11-dehydrothromboxane B₂ predicted a nearly two-fold increased risk for myocardial infarction (MI), stroke or cardiovascular death in individuals on either aspirin or aspirin and clopidogrel therapy (10, 11). In another study, the Verify Now Aspirin assay or traditional platelet aggregometry with arachidonic acid and ADP predicted an increased risk for MI, stroke, or death (13). Though, several smaller studies have addressed ischemic events and stent thrombosis versus no stent thrombosis during clopidogrel treatment, a recent large-scale study (n = 1608) using the Multiplate system identified a nearly 11-fold increased risk of definite stent thrombosis in low responders to clopidogrel (8, 14, 15, 25, 43).

Discussion

The potential relationship between variability to antiplatelet therapy and clinical outcome is not understood due to a lack of an accepted definition of resistance. As new functional and biochemical assays become available for research, the understanding of platelet physiology and the pharmacodynamics associated with antiplatelet drugs is increasing. Accompanying this knowledge is the development of new antiplatelet therapies as well as additional tests to monitor platelet response (3). Insufficient exposure to an antiplatelet drug taken to prevent atherothrombotic disease entails increased risk for an adverse event whereas overexposure to an antiplatelet drug increases the risk for adverse effects such as fatal bleeding (51). Drug therapy inherently depends on absorption, metabolism and excretion. The variability of response to a given drug is not a surprise given that environment, genetics, and disease can affect the drug’s disposition. In fact, most major drugs are effective in only 25–60 percent of patients (45). Exacerbating the issue is the complexity with which platelets contribute to thrombus formation.

The ability to monitor platelet response to aspirin and clopidogrel therapy may have a major impact on patient care resulting in a significant decrease in morbidity and mortality. Clinical evidence is culminating to sup-
Tab. 2  Commercially available tests for monitoring anti-platelet agents

<table>
<thead>
<tr>
<th>trade name</th>
<th>manufacturer</th>
<th>test principle</th>
<th>sample</th>
<th>agonist</th>
<th>instrumentation</th>
<th>registration</th>
</tr>
</thead>
<tbody>
<tr>
<td>aggregometry</td>
<td>various</td>
<td>in vitro platelet to platelet aggregation measured turbidometrically or by electrical impedance</td>
<td>plasma or whole blood</td>
<td>AA, ADP, collagen, TRAP</td>
<td>aggregometer</td>
<td>FDA cleared and CE marked</td>
</tr>
<tr>
<td>Multiplate (MEA)</td>
<td>Dynabyte</td>
<td>in vitro platelet to platelet aggregation measured by electrical impedance on multiple electrodes</td>
<td>whole blood</td>
<td>AA, ADP, collagen, TRAP, ristocetin</td>
<td>Multiplate aggregometer</td>
<td>CE marked</td>
</tr>
<tr>
<td>Impact and Impact-R</td>
<td>Diamed</td>
<td>in vitro shear induced platelet adhesion</td>
<td>endogenous adhesive proteins</td>
<td>Impact or Impact-R instrument</td>
<td>CE marked</td>
<td></td>
</tr>
<tr>
<td>PFA-100</td>
<td>Dade-Behring</td>
<td>in vitro aperture closure due to platelet plug formation under shear stress</td>
<td>collagen/Epi; collagen/ADP</td>
<td>PFA-100</td>
<td>FDA cleared and CE marked</td>
<td></td>
</tr>
<tr>
<td>Plateletworks</td>
<td>Helena Laboratories</td>
<td>in vitro platelet to platelet aggregation measured by single platelet counting</td>
<td>AA, ADP, collagen</td>
<td>CBC analyzer</td>
<td>FDA cleared and CE marked</td>
<td></td>
</tr>
<tr>
<td>Verify Now</td>
<td>Accumetrics</td>
<td>in vitro platelet aggregation to fibrinogen coated beads</td>
<td>AA, ADP, TRAP</td>
<td>Ulategra RPFA</td>
<td>FDA cleared and CE marked</td>
<td></td>
</tr>
<tr>
<td>Platelet Mapping</td>
<td>Haemoscope</td>
<td>in vitro platelet contribution to clot strength measured by thrombo- elastograph</td>
<td>AA, ADP</td>
<td>thrombo- elastograph</td>
<td>FDA cleared and CE marked</td>
<td></td>
</tr>
<tr>
<td>VASP</td>
<td>BioCytex</td>
<td>determination of phosphorylation of protein directly linked to activity of P2Y12 receptor</td>
<td>ADP with and without PGE1</td>
<td>flow cytometer</td>
<td>CE marked</td>
<td></td>
</tr>
<tr>
<td>Aspirinworks</td>
<td>Corgenix</td>
<td>immunological determination of urinary 11-dehydrothromboxane B2, a metabolite of in vivo TXA2 generation</td>
<td>urine</td>
<td>not applicable</td>
<td>microplate reader or automated microplate processing system</td>
<td>FDA cleared and CE marked</td>
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</table>

AA: arachadonic acid; ADP: adenosine diphosphate; TRAP: thrombin receptor activating peptide; MEA: multiple electrode aggregation; PFA: platelet function analyzer; CBC: complete blood count; RPFA: rapid platelet function analyzer; VASP: vasodilator stimulated phosphoprotein

The use of the term resistance should not be taken lightly as it may have detrimental effects when misinterpreted. Falsey identified, it may infer an increased risk of thrombosis if treatment were discontinued or alternatively an increased risk of haemorrhage if dosage were mistakenly increased.

Additional studies are necessary to define the clinical manifestation(s) of resistance, the optimal test(s) necessary to properly diagnose the condition, and the appropriate course(s) of treatment.

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Aspirin and clopidogrel resistance


