

Review Article

A Review of Antiplatelet Drugs, Coronary Artery Diseases and Cardiopulmonary Bypass

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Abstract: Platelets play a major role in the thromboembolic diseases and upon vascular injury, especially arterial vascular injury. These platelets rapidly adhere to the exposed subendothelial area, where they become activated by contacting with stimulants. Antiplatelet therapy remains extremely important in treatment and prophylaxis of arterial thromboembolic disorders such as coronary arterial diseases and stroke. The antiplatelet drugs (APDs) are among the most widely used in the world. Based on the molecular targets, APDs are classified as Thromboxane A2 pathway blockers, ADP receptor antagonists, GPIIa/IIIb antagonists, adenosine reuptake inhibitors, phosphodiesterase inhibitors, thrombin receptor inhibitors, and others. Coronary artery bypass graft (CABG) surgery is an important therapeutic approach to treat coronary artery disease. Long-term success after CABG depends on the patency of the bypass vessels. Since platelets play a crucial role in the pathogenesis of thrombosis in the blood vessels, APDs are broadly used to reduce serious cardiovascular events. Platelets

also are an integral part of inflammation and APDs have demonstrated to reduce the inflammation mediators in the healthy volunteers and coronary artery disease patients; it will be an interesting topic to determine if platelet inhibition will attenuate CPB-induced systemic inflammatory response syndrome. Due to concerns of post-op bleeding with use of APDs, it is a common practice to withhold APDs prior to surgery; however, recent studies have demonstrated that continuation of APDs prior to surgery (even until the day of surgery) does not increase the risk of post-op bleeding. With extensive use of APDs in cardiovascular thromboembolic events, APD resistance becomes problematic in clinical antiplatelet therapy. Since there is no standardized or universal definition available to quantify APDs resistance, a clinically meaningful definition of APD resistance needs to be developed based on data linking laboratory tests to clinical outcomes in patients. **Keywords:** antiplatelet drug, coronary artery diseases, cardiopulmonary bypass, clinical trials, drug resistance, platelet mapping. *JECT. 2010;42:103–113*

Platelets are anuclear cell fragments derived from megakaryocytes that function in the clotting system and circulate in blood for a life span of 6–9 days. Platelets play a major role in the thromboembolic diseases, upon vascular injury, especially arterial vascular injury; platelets rapidly adhere to the exposed subendothelial area, where they become activated by contacting with stimulants like collagen, resulting in the recruitment of additional platelets from circulation to form a stable arterial plug. Platelet plug formation is the primary phase of homeostasis physiologically and

pathologically. It has been widely accepted and confirmed that antiplatelet therapy remains extremely important in treatment and prophylaxis of arterial thromboembolic disorders such as coronary arterial diseases (CAD) and stroke. Antiplatelet drugs (APDs) are a large group of powerful pharmacological agents that interfere with the physiologic activities of platelets and prevent the formation of blood clots. APDs are widely prescribed for the patients with history of CAD, heart attack, angina, atrial fibrillation, and so forth. APDs are also used after procedures of angioplasty, stent placement, and coronary artery bypass grafting (CABG) to prevent stenosis and clotting. This review discusses the category, mechanisms, and clinical trials of common APDs that are used in the treatment of CAD. We also discuss perioperative use of APDs in the CABG procedures, association of platelet inhibition with cardiopulmonary bypass-induced inflammation, and APDs resistance.

Received for publication May 12, 2009; accepted January 16, 2010.
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The senior author has stated that authors have reported no material, financial, or other relationship with any healthcare-related business or other entity whose products or services are discussed in this paper.

CATEGORY, MECHANISM, AND CLINICAL TRIALS

There are three principle phases in thrombus formation: platelet adhesion, platelet activation, and platelet aggregation. All of antiplatelet strategies are designed to work on these three stages; the established APDs accordingly interfere with one or more of three stages, however these different APDs have different molecular targets. Here the classification of APDs is based on their molecular targets/receptors on the platelet (see Figure 1), and the representative drugs from each category are also listed (see Table 1).

Thromboxane A2 Pathway Blockers

Thromboxane A2 (TXA₂) is a potent agonist of platelet activation and vasoconstriction. Cyclooxygenase (COX) is a key enzyme in the conversion of arachidonic acid to

prostaglandin H2 that leads to the production of TXA₂. After binding to its receptor on platelet, TXA₂ induces phospholipase C-β (PLC) activation, resulting in intracellular Ca²⁺ increase and subsequent platelet activation. Acetylsalicylic acid (ASA, Aspirin) blocks TXA₂ synthesis by irreversible acetylating Ser-529 and Ser-516 in COX1 and COX2, respectively. Valdecoxib, parecoxib, celecoxib, and rofecoxib are members of COX2 selective inhibitors that are under investigation in the patients with cardiovascular events (1). Because platelets are anucleate and no new COX can be generated, a single dose of Aspirin has a permanent platelet inhibition effect that lasts the lifespan of the platelets (6–9 d) despite its relatively short plasma half-life (5–20 minutes). Aspirin was first introduced into market under the trademark Aspirin® in the year 1899. Since then it has attained a leading position world-wide in

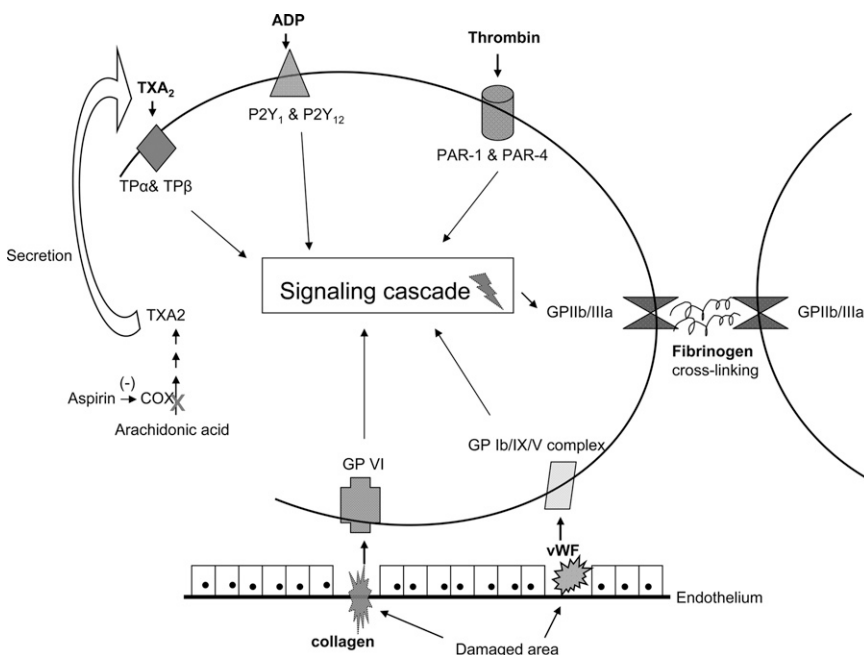


Figure 1. Schematic diagram of major receptors/molecular targets and corresponding ligands on the platelet surface involving the platelet activation. TP, TXA₂ receptor.

Table 1. Summary of category, molecular targets, and representatives of antiplatelet drugs.

Category	Molecular Targets	Representative Drugs or Compounds
1. TXA ₂ pathway blockers	COX-1 and COX-2 TXA ₂ receptor	Aspirin (COX1 and COX2) Specific COX2 inhibitors: Valdecoxib, Parecoxib, Celecoxib, and Rofecoxib TXA ₂ receptor antagonist: Ridogrel, Ramatroban (BAY u 3405), and S18886
2. ADP receptor antagonist	P2Y1 and P2Y12	Clopidogrel (Plavix), Ticlopidine (Ticlid), Prasugrel, and Ticagrelor (AZD6140)
3. GPIIa/IIIb antagonist	GPIIa/IIIb (fibrinogen receptor)	Abciximab (ReoPro), Eptifibatide (Integrilin), Tirofiban (Aggrastat)
4. Adenosine reuptake inhibitor	Adenosine deaminase and cyclic GMP phosphodiesterase (type V)	Dipyridamole (Persantine) Aggrenox (aspirin/dipyridamole)
5. Phosphodiesterase inhibitors	Phosphodiesterase (type III)	Cilostazol (Pletal)
6. Thrombin receptor inhibitor	PAR1 and PAR4	E5555 (PAR1) and sch530348 (PAR1)
7. Others	Blocking the vWF binding to collagen P-selectin GPVI (collagen receptor)	C1qTNF-related protein-1 (CTRP-1) P-selectin antagonist: PSI421 and PSI-697 EXP3179 and trowaglerix

the prescription-free therapy of painful, inflammatory, and feverish states, and entitled Wonder (Miracle) Drug of the 20th Century. In the 1970s, Aspirin was first found to also have antiplatelet or "anti-clotting" effect and since then has been used in long-term, low doses to prevent heart attacks, strokes, and blood clot formation in people at high risk for developing blood clots (2). It has also been established that low doses of aspirin may be given immediately after a heart attack to reduce the risk of a subsequent heart attack (3). No other pharmacologic agent can challenge the risk-benefit or cost-benefit ratios of aspirin therapy. Aspirin is the mandatory treatment for secondary prevention of cardiovascular events. Although there is evidence that high-dose aspirin (500 mg) additionally inhibits thrombin generation and erythrocyte-mediated platelet activation, aspirin has a rather weak effect on platelets (4,5). Indeed, since aspirin targets only the TXA₂-dependent platelet activation amplification loop, several other stimuli, such as shear forces, and more potent platelet agonists (e.g., thrombin or collagen) can actually bypass the defect in TXA₂-production and activate platelets. Recently a randomized trial of low-dose aspirin (100 mg every other day) in the primary prevention of cardiovascular disease in women (45 years of age or older) demonstrated that aspirin lowered the risk of stroke but did not affect the risk of myocardial infarction or death from cardiovascular causes, leading to a non-significant finding compared to placebo (6). Therefore co-use of aspirin and other APDs with different targets, especially clopidogrel, an adenosine diphosphate (ADP) receptor inhibitor, has been shown to reduce ischemic complications in patients presenting with acute coronary syndrome and has been the standard of care in patients with acute coronary syndromes (7,8). Ridogrel, ramatroban (BAY u 3405), and S18886 are TXA₂ receptor antagonists that inhibit the interaction of TXA₂ and its precursors, prostaglandins G₂ and H₂. Ridogrel has been studied primarily as an adjunctive agent to thrombolytic therapy in acute myocardial infarction (AMI). Despite positive results from initial pilot studies, the largest clinical study, the Ridogrel versus Aspirin Patency Trial (RAPT), failed to demonstrate any advantage with this agent over aspirin (9). In the study of 907 patients with AMI, there was no difference in the primary end point of infarct vessel patency rate between those randomized to ridogrel (72.2%) or aspirin (75.5%) (9). As such, there are currently no clinical indications for preferential use of ridogrel over aspirin.

ADP Receptor Inhibitors

Platelets have three purinergic receptors, two of which, P2Y₁ and P2Y₁₂, are receptors for ADP. The binding of ADP to the G protein-coupled receptors P2Y₁₂ and P2Y₁ initiates platelet aggregation, but, more importantly, it further amplifies platelet response to other stimuli such as TXA₂ and thrombin (10). The ADP receptor inhibitors exert

their major effect by specifically inhibiting the P2Y₁₂ subtype. The current three common compounds, clopidogrel (Plavix), ticlopidine (Ticlid), and prasugrel are prodrugs that need to be metabolized by the liver cytochrome P450-dependent pathway to the active metabolite, which results in a delayed onset of action. The short-living active compounds have a free thiol that forms a disulfide bridge with both extracellular cysteines Cys17 and Cys270 of P2Y₁₂ when platelets reach the liver, leading to irreversible inhibition of the ADP-receptor that lasts for the lifetime of the platelet. A large randomized clinical trial to evaluate the effectiveness and safety of clopidogrel was the CAPRIE (Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events) study, which was a secondary prevention study comparing clopidogrel (75 mg/d) versus aspirin (325 mg/d) therapy in patients with recent myocardial infarction (MI), ischemic stroke, or symptomatic peripheral arterial disease. In this study of 19,185 subjects, clopidogrel use was associated with an 8.7% relative risk reduction for the composite outcome of vascular death, MI, or stroke (11). Clopidogrel also reduced rehospitalization for ischemic events to a greater extent than aspirin (12). Clopidogrel was shown to be similar to aspirin in safety as there were no major differences in adverse events between the two therapies. Dual drug therapy with aspirin and clopidogrel has been studied and demonstrated to be superior to aspirin alone in the treatment of patients with acute coronary syndromes and after coronary stenting (7,8,13); however some data do not support the beneficial effect of this dual antiplatelet therapy (13). Therefore large clinical trials with standardized laboratory methods and well-defined protocols are necessary to determine whether common features exist in patients with suspected hyporesponsiveness or nonresponsiveness (resistance) to antiplatelet therapy, and to validate the clinical relevance of response variability (14).

Glycoprotein IIb/IIIa Antagonist

The Glycoprotein (GP)IIb/IIIa is a fibrinogen receptor that is vital to the process of platelet activation and aggregation. The importance of the GPIIb/IIIa receptor in the process of hemostasis and thrombosis is illustrated by the bleeding disorder Glanzmann thrombasthenia, which is due to deficient GPIIb/IIIa receptors on the patients' platelets. The GPIIb/IIIa receptor, in response to intracellular reactions, undergoes a conformational change allowing it to bind its most important ligand, fibrinogen. Once this occurs, platelet aggregation occurs by the cross-linking of separate platelets with fibrinogen. Since the fibrinogen-GPIIb/IIIa interaction is the major final step in platelet aggregation and GPIIb/IIIa is only expressed on platelets, it is clear that the development of GPIIb/IIIa antagonists is an attractive strategy for antiplatelet therapy. The GPIIb/IIIa receptor antagonists provide potent antiplatelet activity, much more than that seen with aspirin or the

oral ADP receptor antagonists clinically evaluated to date. Abciximab is the chimeric Fab fragment of the murine anti-human GPIIb/IIIa monoclonal antibody developed in 1985 (15). Abciximab is recommended as adjunct therapy in acute coronary syndromes and percutaneous coronary intervention (PCI) (16–18). A meta-analysis of clinical trials on the use of abciximab in PCI has shown that abciximab treatment resulted in a 20% decrease in all-cause mortality during long term follow-up (16). In the ADMIRAL (Abciximab Before Direct Angioplasty and Stenting in Myocardial Infarction Regarding Acute and Long Term Follow-Up) trial of 300 patients, those randomized to abciximab plus PCI as compared with PCI alone had a significant 59% reduction in the rate of death, recurrent MI, or urgent target vessel revascularization at 30 days (19). The majority of trials indicate a significant benefit with use of adjunctive GPIIb/IIIa inhibitor therapy during primary PCI reperfusion for acute MI (20). In contrast to the observed efficacy of intravenous GPIIb/IIIa inhibitors, the trials with oral GPIIb/IIIa inhibitors have failed to demonstrate any benefit in the management of acute coronary syndromes and PCI (21).

Adenosine Reuptake Inhibitor

Dipyridamole (Persantine) inhibits the uptake of adenosine into platelets *in vitro* and *in vivo*. In addition to its inhibition of adenosine deaminase resulting in increased concentrations of adenosine, dipyridamole has also demonstrated ability to selectively inhibit the cyclic guanosine monophosphate (GMP) phosphodiesterase type V enzyme, thereby enhancing the antiplatelet effects of the NO/cyclic GMP signaling pathway (22). Although primarily studied for use in cerebrovascular disease, there have been investigations of dipyridamole with or without aspirin as a therapeutic modality for secondary prevention of MI. In the Persantine-Aspirin Reinfarction Study (PARIS) of more than 2000 patients with history of MI, no further benefit in death or MI prevention was gained with the addition of dipyridamole to aspirin therapy (23,24). In the subsequent Persantine-Reinfarction Study Part II (PARIS II) trial, dual therapy with dipyridamole and aspirin significantly reduced the composite end point of MI or death at 1 year as compared with placebo (25). The beneficial effect of persantine plus aspirin compared with placebo for coronary incidence tended to be greater for the following groups of patients: those who had a non-Q wave infarct; those who were not taking digitalis; those who were receiving beta-receptor blocking drugs at baseline; those who were in New York Heart Association functional class I; those who had had only one myocardial infarction; or those who were enrolled in the study early, that is within 85 days of the qualifying myocardial infarction (25). The randomized controlled trial ESPRIT (Aspirin plus dipyridamole versus aspirin alone after cerebral ischemia of

arterial origin), combined with the results of previous trials, provided sufficient evidence to prefer the combination regimen of aspirin plus dipyridamole over aspirin alone as antithrombotic therapy after cerebral ischemia of arterial origin (26).

Phosphodiesterase Inhibitors

Cilostazol (Pletal) reversibly inhibits platelets via its selective antagonism of the cyclic nucleotide phosphodiesterase type 3 enzyme, and it is also known to inhibit adenosine uptake. Its ability to inhibit platelets *in vivo*, however, is uncertain. Unlike conventional antiplatelet agents, cilostazol has several favorable properties in reducing restenosis. Besides the vasodilatory effect, cilostazol directly inhibits smooth muscle proliferation and may enhance re-endothelialization after percutaneous transluminal coronary angioplasty (PTCA). Although the magnitude of prevention of restenosis may differ with the PTCA device used, cilostazol appears quite promising as a pharmacologic treatment adjunct to PTCA (27). In the Cilostazol for Restenosis Trial (CREST), 705 patients undergoing PCI with stent implantation were randomized to cilostazol (100 mg twice a day) or placebo in addition to aspirin and clopidogrel (75 mg daily for 1 month) therapy. There was a significant 36% reduction in the rate of restenosis in those randomized to cilostazol as compared with placebo ($p = .002$) (28). Meanwhile restenosis was significantly lower in cilostazol-treated diabetics (17.7% versus 37.7%, $p = .01$) and in those with small vessels (23.6% versus 35.2%, $p = .02$), long lesions (29.9% versus 46.6%, $p = .04$), and left anterior descending coronary artery site (19.3% versus 39.8%, $p = .001$) (28). A recent study demonstrated that dual therapy of aspirin and cilostazol is much more effective than aspirin alone in reducing platelet aggregation (collagen- and arachidonate-induced aggregation, $p < .0001$) in patients after off pump CABG (OPCAB) (29). This combination therapy may represent a new therapeutic option for an anti-thrombotic regimen in patients after OPCAB.

Thrombin Receptor Inhibitor

Thrombin is one of the most potent platelet activators *in vivo* through its interaction with the G-protein coupled protease-activated receptors (PARs) on the platelet surface. The thrombin receptors (PAR1 and PAR4) on the human platelet surface are well characterized (30), PAR1 and PAR4, both of which are G protein-coupled receptors. PAR1 is a high-affinity receptor and the major effector of thrombin signaling in the platelet, whereas PAR4 supplements its actions in the later stages of platelet activation. There is much interest in the clinical development of both PAR1 and PAR4 antagonists as novel antiplatelet therapeutic modalities. Recent studies suggest that simultaneous PAR1 and PAR4 antagonism is synergistic and provides more effective inhibition of thrombin-induced platelet activation than with

either PAR1 or PAR4 antagonism alone (31). Two of the orally administered PAR1 antagonists currently undergoing evaluation in phase II studies are E5555 and SCH530348 (32). A randomized, double-blind, placebo-controlled study of the safety and tolerability of E5555, and its effects on markers of intravascular inflammation in subjects with coronary artery disease, with an expectant enrollment of 600 subjects, will evaluate the efficacy and safety of E5555 in patients with CAD (phase II, Study #NCT00548587). An additional on-going trial, TRACER (Trial to Assess the Effects of SCH 530348 in Preventing Heart Attack and Stroke in Patients with Acute Coronary Syndrome) is designed to determine whether SCH 530348, when added to the existing standard of care (e.g., aspirin, clopidogrel) for preventing heart attack and stroke in patients with acute coronary syndrome, will yield additional benefit over the existing standard of care in preventing heart attack and stroke (phase III, Study #NCT00527943). This study is also designed to assess risk of bleeding with SCH 530348 added to the standard of care versus the standard of care alone.

Other Novel APDs

There have been studies of the new targets aimed at interfering the critical interactions between platelets and injured subendothelial area to prevent platelet adhesion altogether. One agent undergoing investigation is the collagen inhibitor, C1qTNF-related protein-1 (CTRP-1), which has been shown to inhibit platelet aggregation by blocking the ability of von Willebrand factor (vWF) to bind to collagen, thereby interrupting platelet adhesion and thrombogenesis (33). Platelet collagen receptor, glycoprotein VI (GPVI), plays a critical role in acute coronary thrombosis. Bigalke and colleagues reported that surface expression of GPVI is enhanced in patients with acute coronary syndrome and indicates an imminent acute coronary event before irreversible myocardial necrosis is evident (34). High GPVI levels are associated with increased residual platelet aggregation despite antiplatelet therapy. Obviously targeting the GPVI-collagen interaction has clear antithrombotic potential and clinical trials are needed to validate this therapeutic strategy. P-selectin is a member of the selectin family of adhesion molecules. P-selectin participates in the initiation of platelet aggregation by stabilizing GPIIb/IIIa-fibrinogen interactions and by increasing the secretion of TXA₂, suggesting that P-selectin may be an attractive antiplatelet target. P-selectin inhibition has been evaluated as a therapy for prevention and treatment of venous thrombosis. A novel oral small-molecule inhibitor of P-selectin, PSI-697, was demonstrated to reduce thrombus growth and decrease neo-intima formation in rat model (35). In addition, there were reports showing that some calcium channel blockers and herb ingredients have the ability of antiplatelet effects (36,37), but their clinical antithrombotic outcomes are uncertain and needed to be determined.

PERI-OPERATIVE USE OF APDS IN CABG PROCEDURES

CABG is an important therapeutic approach to treat coronary artery disease. CABG is the most frequent cardiac surgery procedure, with nearly 1 million operations conducted per year worldwide, and its growth is likely to accelerate, given the aging of the world population and the increasing availability of this therapy in developing countries. Although there have been substantial advances in surgical techniques, myocardial preservation, and hemodynamic monitoring, complication rates continue to be problematic. Indeed, the long-term success after CABG depends on the patency of the bypass vessels that is mainly influenced by the type of the graft used, but also by the quality of the distal vessel into which the graft is placed. Since platelets play a crucial role in the pathogenesis of thrombosis in the blood vessels, APDs are broadly used to reduce serious cardiovascular events especially coronary artery events. In general, there is general consensus about long-term aspirin use in reducing the risk of death, MI, and stroke in patients at high risk of occlusive disease (38–40). A recent investigation of in-hospital medication use among 2389 consecutive patients who underwent CABG demonstrated that approximately 95% of CABG patients received aspirin at least once during their hospitalization; 25% of patients received aspirin on the day of admission, and less than 50% received aspirin on the day of surgery (41). The use of aspirin increased following CABG, reaching 80.4% during the postsurgical period, indicating that aspirin may have been withheld prior to surgery due to concerns of bleeding complications. The most common dosage of aspirin was 325 mg (86.0% of aspirin prescriptions).

The fate of coronary artery bypass grafts depends on many factors, including technical faults in harvesting, handling, and fashioning the conduits; thrombosis, myointimal hyperplasia, fibrosis; and a rapidly progressing variety of atherosclerosis. Coronary bypass graft disease and occlusion are common after coronary artery bypass grafting and increase with time. There are major determinants of clinical prognosis, specifically measured by reoperation rate and survival. Vein graft patency and disease have been shown to be closely related to long-term survival after CABG (42). The meta-analysis of the antiplatelet trialists' collaboration revealed that antiplatelet therapy (chiefly aspirin alone or aspirin plus dipyridamole) greatly reduces the risk of vascular occlusion in a wide range of patients at high risk of this complication (43–45). Additionally, a large-scale, multi-centered trial involving 5065 patients undergoing coronary bypass surgery has demonstrated that aspirin therapy (aspirin up to 650 mg within 48 hours after revascularization) was associated with a 48% reduction in the incidence of myocardial infarction, and multivariate

analysis demonstrated that no other factor or medication was independently associated with reduced rates of these outcomes, and that the risk of hemorrhage, gastritis, infection, or impaired wound healing was not increased with aspirin use (40).

There is still a discrepancy regarding discontinuation of antiplatelet drugs before CABG surgery. It is not uncommon for aspirin and clopidogrel to be withheld before CABG surgery because it is thought to increase post-operative bleeding and the risk associated with it (46–48). However, recent studies do not support withdrawal of antiplatelet drugs before surgery. Kamran et al. reported that use of aspirin until the date of surgery does not increase the risk of post-op bleeding. In contrast, significant reduction in the bleeding was found in the group that aspirin was not withheld prior to surgery ($p = .004$ in 2 hours post-op and $p = .043$ in 28–76 hours post-op) (49), however the author did not mention the dosage of aspirin used in the trial. Sun and colleagues pointed out that dose of aspirin less than 325 mg/day did not appear to increase post-op bleeding (50). In addition, pre-operative aspirin users were 27% less likely to experience a fatal outcome than nonusers by univariate analysis (odds ratio = .73, 95% confidence interval [.54, .97]; $p = .03$) without significant increase in hemorrhage, blood product requirements, or related morbidities (51). Although withdrawal of clopidogrel was recommended at least 5 days prior to elective CABG surgery (52), a recent study that involved 217 consecutive patients with CABG demonstrated that pre-op use of clopidogrel with low dose of aspirin within 5 days prior to surgery was not associated with an increased risk of excessive post-op bleeding and transfusion requirements (53). These results are important because aspirin alone may not be sufficient in inhibition of platelet aggregation and TXA_2 formation early after CABG (54); also the maintenance of combined antiplatelet therapy until the surgery may allow avoiding adverse coronary events before surgery.

Fox et al. reported that in patients undergoing CABG and continuing clopidogrel (and aspirin) within 5 days before CABG, there is a non-significant trend of 1 additional patient per 100 that experiences life-threatening bleeding, and an additional 2 patients per 100 that experiences a major bleed compared to aspirin only group; therefore, the benefits of starting clopidogrel on admission appear to outweigh the risks, even among those who proceed to CABG during the initial hospitalization (7). For those patients undergoing elective CABG operations after recent clopidogrel exposure, a strict algorithm-driven treatment of bleeding was applied to minimize the transfusion (55). In addition, Aprotinin was reported to decrease the post-op bleeding and numbers of transfusions in patients on clopidogrel undergoing CABG (56).

PLATELET INHIBITION AND CARDIOPULMONARY BYPASS

It is well documented that the use of cardiopulmonary bypass (CPB) is associated with the development of significant systemic inflammatory response syndrome (SIRS) (57,58) which can affect patient outcomes. SIRS is characterized by high cardiac output, low mean and systolic blood pressure, low systemic vascular resistance, and a core temperature greater than 38°C or less than 36°C in the immediate postoperative period. Originally, this physiologic response was attributed only to the contact of blood components to the large foreign surface area of the CPB circuit; however, today it is believed to be mediated by a complex relationship between activated neutrophils, platelets, complement activation, cytokines, coagulation, fibrinolytic, and kallikrein cascades. Some techniques and therapies have been shown to reduce the effects of SIRS to CPB such as minimal extracorporeal circulation systems (Synergy) (59), hemoconcentrators (60), and leukocyte depletion filters (61). In addition, Sobieski and colleagues reported that a single dose of dexamethasone (100 mg) significantly reduced IL-6 ($p = .0005$) levels associated with CPB (62).

Platelets are not only involved in homeostasis but also directly initiate an inflammatory response of the vessel wall. Studies have demonstrated that platelets are an integral part of inflammation and can be potent effectors of the innate immune response (63). The participation of platelets in immunity is accomplished by their release of inflammatory mediators such as P-selectin, IL-6, PF4, IL-7, IL-8, histamine, serotonin, CD40 ligand (CD40L, also called CD154), TGF- β , TXA_2 , PAF, etc. upon activation. Of these mediators, CD40L is a molecule that is drawing more and more research attention because of its important role in neutrophil adhesion and transmigration at injured endothelium involving platelets. CD40L, a transmembrane protein structurally related to the cytokine TNF- α , was originally identified on stimulated CD4 + T cells. The interaction between CD40L (T cells) with CD40 (B cells) is of paramount importance for the development and function of the humoral immune system. It was reported that platelets express CD40L as well, within seconds, of activation *in vitro* and in the process of thrombus formation *in vivo* (64). Like TNF- α , CD40L on platelets induces endothelial cells to secrete chemokines and to express adhesion molecules, thereby generating signals for the recruitment and extravasation of leukocytes at the site of injury. The CD40L expressed on stimulated platelets is subsequently cleaved, which generates a soluble hydrolytic fragment termed sCD40L. Essentially, all of the sCD40L generated during the clotting of whole blood is derived from platelets. sCD40L appears to be involved in both thrombosis and inflammation (65). Indeed, elevated levels of sCD40L have been documented in several thrombotic

and inflammatory conditions, including acute coronary syndromes and peripheral arterial occlusive disease (66). Antiplatelet drugs including aspirin and clopidogrel have demonstrated to reduce the CD40L in the healthy volunteers and CAD patients (67,68).

It has been shown that CPB causes an increase in the concentration of plasma sCD40L (69). Plasma levels of sCD40L increased greater than 1.7-fold ($p = .001$) within 1 hour on CPB and increased even further to 3.7-fold ($p = .03$) 2 hours post-operatively. To date, there are no published studies investigating the effects of antiplatelet drugs on sCD40L elevation associated with CPB. If antiplatelet drugs are determined to attenuate sCD40L elevation associated with CPB, then this may potentially provide us with a new clinical therapy for CPB induced inflammation.

APDS RESISTANCE AND PLATELET MAPPING

There is no standardized or universal definition available to quantify APDs resistance (or hyporesponsiveness or nonresponsiveness) even though APD "resistance" has received increasing attention over recent years. Before discussing APD resistance, it is necessary to understand that the response to most medication, including APDs, is highly variable between individuals. Optimal antiplatelet therapy should be considered based on the individualized patients; secondly, inhibition of platelet aggregation as measured in vitro does not necessarily translate into prevention of thrombosis in vivo and it is difficult to investigate in which capacity the laboratory resistance corresponds to the clinical resistance. Most studies of APD resistance focused on the resistance of aspirin and clopidogrel. Although the term, GP IIb/IIIa antagonist resistance, has not been used so far in the literature, a study involved with 500 patients undergoing PCI with the planned use of a GP IIb/IIIa inhibitor showed substantial variability in the degree of platelet inhibition and there is in vitro evidence of abciximab resistance (70).

Generally three aspirin resistance (or aspirin nonresponsiveness) categories have been proposed: 1) aspirin fails to inhibit the platelet function in vivo or in vitro. Currently there are the following common methods that are used for the evaluation of aspirin resistance: bleeding time; platelet light transmittance aggregometry (LTA); point-of-care assays including PFA-100 (platelet function analyzer) and Ultegra Rapid Platelet Function Assay-ASA (RPFA-ASA); and miscellaneous techniques such as flow cytometry and thrombelastography (TEG). The bleeding time is a highly inaccurate and poorly reproducible technique, which is dependent on several variables, including platelet function, platelet count, plasma factors, red blood cells, and the vessel wall. Therefore, the bleeding time is an improper method to measure platelet inhibition by aspirin. The gold

standard for evaluating platelet responsiveness is LTA, which measures the increase in light transmission through a platelet suspension that occurs when platelets are aggregated by an agonist. However, LTA is a time-consuming technique and requires a specialized laboratory. In addition, the results obtained within one laboratory can barely be compared with those obtained in a different laboratory because of lack of standardization. TEG Platelet Mapping seems a promising technique to measure the platelet inhibition by aspirin but has its limitation (see the following context). 2) Aspirin fails to prevent vascular occlusion. Actual failure of aspirin to prevent clinical events associated to vascular occlusion should be precisely termed as aspirin treatment failure instead of resistance, as we know, vascular occlusion is a complex event involved with multifactorial mechanism. Aspirin only has one pathway to block platelet aggregation (by inhibiting the COX-1 that leading to reduce TXA₂ synthesis), and platelet aggregation is only one of several mechanisms that regulate the thrombus formation. Furthermore, thrombus formation is not only one mechanism that develops the vascular occlusion. It would be unreasonable to expect aspirin, or any APD, to prevent clinical vascular events in all patients at risk. In addition, variability of response to aspirin treatment exist clinically; some clinical trials have shown that women are more likely to be aspirin-resistant than men (71). Additional factors such as the age, smoking, and diabetes may influence the patients' response to aspirin treatment (72,73). Therefore, it is inappropriate to term aspirin resistance based on pure clinical outcomes. 3) Aspirin fails to inhibit the TXA₂ formation: Since the antiplatelet effect of aspirin is caused by the inhibition of COX-1, TXA₂ is a particularly suitable parameter to assess the antiplatelet effect of aspirin. TXA₂ rapidly hydrolyzes to the stable metabolite, TXB₂, which is the most specific indicator to measure the pharmacological effect of aspirin. Based on the available techniques such as enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay, the acceptable definition of aspirin resistance should rely on the demonstration of an insufficient inhibition of TXA₂ production. Although some authors refer to failure of aspirin to inhibit TXA₂ production with the term "true" aspirin resistance, some cells (endothelial cells and monocytes) can provide prostaglandin H₂ (PGH₂) to platelets (bypassing COX-1) and synthesize their own TXA₂, which limits the specificity for aspirin resistance (74).

Clopidogrel (like ticlopidine) is a prodrug that can be metabolized by hepatic cytochrome P450 into active metabolites, which irreversibly inhibits binding of ADP to the P2Y₁₂ receptor on the platelet. There are two types of ADP receptors on the platelet, P2Y₁ and P2Y₁₂; stimulation of P2Y₁ receptor mediates shape change and transient reversible aggregation, and stimulation of P2Y₁₂ receptor induces lasting aggregation and decrease in cAMP. The

extent of the platelet aggregation response in vitro to ADP has been used to define “clopidogrel resistance” in the majority of published studies thus far. Because the extent of residual, P2Y1-dependent platelet aggregation induced by ADP varies widely among patients with congenital P2Y12 deficiency or normal subjects in whom P2Y12 function had been completely blocked in vitro by saturating concentrations of specific antagonists, ADP induced platelet aggregation may not be the most suitable test to measure the individual response to clopidogrel.

The following potential factors may contribute to the aspirin and clopidogrel mechanism of hyporesponsiveness or nonresponsiveness: 1) Decreased bioavailability: bioavailability is decreased due to noncompliance, underdosing, or poor absorption (enteric-coating aspirin). 2) Interaction with other drugs: Ibuprofen prevents aspirin from accessing at Serine530 site of COX-1; co-administration of clopidogrel with atorvastatin will competitively inhibit clopidogrel activation by interfering with the hepatic P450 enzyme system (75,76). 3) Platelet function changes: platelet turnover is accelerated with introduction into bloodstream of newly formed, drug-unaaffected platelets; stress-induced COX-2 activation in platelets (aspirin) or increased platelet sensitivity to ADP or collagen has been reported (77). 4) Trans-cellular formation of TXA₂ by aspirinated platelets from PGH₂ released by endothelial cells and monocytes or TXA₂ production by other types of cells was also reported (78). 5) Genetic polymorphisms: patients response to aspirin and clopidogrel may in part reflect variation in COX enzyme (COX1–3), glycoproteins (GPIb alpha, GPIa/IIa, GPIIb/IIIa), ADP receptor (P2Y1 and P2Y12), and cytochrome P450 system (CYP2C9, CYP3A4 and CYP3A5) genotypes (79).

The TEG Platelet Mapping assay is a novel assay which measures the percentage of platelet inhibition of aspirin and ADP antagonists like clopidogrel. It relies on evaluation of clot strength as maximal amplitude to enable a quantitative analysis of platelet function. The maximal hemostatic activity is measured by a kaolin activated whole blood sample treated with citrate. Normally activation of either the platelet ADP or TXA₂ receptor results in activation of the platelet GP IIb/IIIa receptor, with resulting platelet activation. However, conventional TEG is mainly dependent on direct thrombin activation of the GP IIb/IIIa receptor. This bypasses the less-potent platelet activators ADP and TXA₂, making it unlikely to assess the contribution to platelet activation (80). Platelet Mapping is designed specifically to overcome this problem and enables assessment of platelet inhibition secondary to inhibition of the platelet ADP and TXA₂ receptors. Tantry et al. applied TEG Platelet Mapping to study the prevalence of aspirin resistance in six healthy subjects and 223 patients with long-term daily aspirin treatment. They found that only one of 223 patients (.4%) was resistant to aspirin

treatment. Aspirin resistance is rare in compliant patients with coronary artery disease when assessed by methods directly dependent on platelet COX-1, therefore aspirin resistance may be overestimated by previous reports using nonspecific laboratory measurements that do not isolate aspirin’s primary target, platelet COX-1 (81). Bochsén et al. studied 43 healthy volunteers to determine the variability of platelet inhibition in response to ADP and arachidonic acid. The results demonstrated a high variability in ADP receptor inhibition, which may explain the differences in response to ADP inhibitor like clopidogrel (82). Alstrom et al. used both Platelet Mapping assay and PFA-100 to compare the platelet inhibition effects of clopidogrel combination with aspirin. Their results showed a significant platelet inhibition with Platelet Mapping but not PFA-100; they suggested that response to APDs for the individual patient with different methods were inconsistent, also further studies are still needed to evaluate how the results correlate to the clinical risk of thrombosis and bleeding (83).

To date, there are still no established guidelines available for the diagnosis and treatment of APD resistance. Theoretically, the adjustment of antiplatelet treatment to an individually diagnosed laboratory or clinical aspirin resistance may include an increased aspirin dose or the addition of (or replacement by) other antiplatelet drugs. Some studies demonstrated that laboratory aspirin resistance can be overcome by a higher dose, both in patients and in healthy subjects; however, it still remains controversial (84–86). Another approach is to combine aspirin with other antiplatelet drugs that may have synergistic effects, such as aspirin and clopidogrel (or dipyridamole or GPIIb/IIIa antagonists); however 47.4% (9/19) of laboratory aspirin-resistant patients (undergoing elective PCI) were identified as clopidogrel-resistant (73,87). In addition, increased antiplatelet therapy may potentially increase the risk of bleeding and other side effects. As mentioned previously, the available clinical data are insufficient to answer whether increasing the dose of aspirin or the addition of another antiplatelet drug has a beneficial impact on the clinical outcome of patients with laboratory aspirin resistance.

FUTURE PERSPECTIVE

The development and improvement of APDs is still an active laboratory and clinical research field. One key challenge for future research is to establish a standardized and validated laboratory or point-of-care tests that are capable of quantifying the antiplatelet effect of aspirin and other APDs. Here is the conclusion quote from the Working Group of Aspirin Resistance of the International Society of Thrombosis and Hemostasis: “*The correct treatment, if any, of aspirin ‘resistance’ is unknown. No published studies*

address the clinical effectiveness of altering therapy based on a laboratory finding of aspirin 'resistance'. Therefore, other than in research trials, it is not currently appropriate to test for aspirin 'resistance' in patients or to change therapy based on such tests. A clinically meaningful definition of aspirin 'resistance' needs to be developed, based on data linking aspirin-dependent laboratory tests to clinical outcomes in patients" (88).

With our understanding of platelet physiology and the role of platelet in the cardiovascular events improving, both new APD with better efficacy and less side-effects and new APD with new targets will be developed and tested. An example is P-selectin, which participates in the initiation of platelet aggregation by increasing the TXA₂ secretion. A recently developed oral antagonist of P-selectin, PSI-421, was demonstrated to promote the resolution of venous thrombosis in the baboon model stasis induced deep vein thrombosis (35). Whereas another antagonist of P-selectin, PSI-697, was shown to reduce the arterial thrombus growth and decrease neo-intima formation in animal model (89). In addition to optimizing the current existing antiplatelet strategies, new fast-acting APD with short half-life should be developed because the short-acting antiplatelet agents are needed and will be convenient when invasive procedures are required (e.g., bypass surgery for acute myocardial infarction). In the near future, it will be exciting to foresee the more promising APDs to be discovered and tested for clinical impacts.

REFERENCES

- Nussmeier NA, Whelton AA, Brown MT, et al. Complications of the COX-2 inhibitors parecoxib and valdecoxib after cardiac surgery. *N Engl J Med*. 2005;352:1081–91.
- Lewis HD Jr, Davis JW, Archibald DG, et al. Protective effects of aspirin against acute myocardial infarction and death in men with unstable angina. Results of a Veterans Administration Cooperative Study. *N Engl J Med*. 1983;309:396–403.
- Julian DG, Chamberlain DA, Pocock SJ. A comparison of aspirin and anticoagulation following thrombolysis for myocardial infarction (the AFTER study): A multicentre unblinded randomised clinical trial. *BMJ*. 1996;313:1429–31.
- Szczeklik A, Krzanowski M, Gora P, Radwan J. Antiplatelet drugs and generation of thrombin in clotting blood. *Blood*. 1992;80:2006–11.
- Santos MT, Valles J, Aznar J, Marcus AJ, Broekman MJ, Safier LB. Prothrombotic effects of erythrocytes on platelet reactivity. Reduction by aspirin. *Circulation*. 1997;95:63–8.
- Ridker PM, Cook NR, Lee IM, et al. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women. *N Engl J Med*. 2005;352:1293–304.
- Fox KA, Mehta SR, Peters R, et al. Benefits and risks of the combination of clopidogrel and aspirin in patients undergoing surgical revascularization for non-ST-elevation acute coronary syndrome: The Clopidogrel in Unstable angina to prevent Recurrent ischemic Events (CURE) Trial. *Circulation*. 2004;110:1202–8.
- Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med*. 2001;345:494–502.
- Randomized trial of ridogrel, a combined thromboxane A₂ synthase inhibitor and thromboxane A₂/prostaglandin endoperoxide receptor antagonist, versus aspirin as adjunct to thrombolysis in patients with acute myocardial infarction. The Ridogrel Versus Aspirin Patency Trial (RAPT). *Circulation*. 1994;89:588–95.
- Storey RF, Sanderson HM, White AE, May JA, Cameron KE, Heptinstall S. The central role of the P(2T) receptor in amplification of human platelet activation, aggregation, secretion and procoagulant activity. *Br J Haematol*. 2000;110:925–34.
- A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). CAPRIE Steering Committee. *Lancet*. 1996;348:1329–39.
- Bhatt DL, Hirsch AT, Ringleb PA, Hacke W, Topol EJ. Reduction in the need for hospitalization for recurrent ischemic events and bleeding with clopidogrel instead of aspirin. CAPRIE investigators. *Am Heart J*. 2000;140:67–73.
- Bhatt DL, Fox KA, Hacke W, et al. Clopidogrel and aspirin versus aspirin alone for the prevention of atherothrombotic events. *N Engl J Med*. 2006;354:1706–17.
- Ferguson AD, Dokainish H, Lakkis N. Aspirin and clopidogrel response variability: Review of the published literature. *Tex Heart Inst J*. 2008;35:313–20.
- Coller BS. A new murine monoclonal antibody reports an activation-dependent change in the conformation and/or microenvironment of the platelet glycoprotein IIb/IIIa complex. *J Clin Invest*. 1985;76:101–8.
- Topol EJ, Lincoff AM, Kereiakes DJ, et al. Multi-year follow-up of abciximab therapy in three randomized, placebo-controlled trials of percutaneous coronary revascularization. *Am J Med*. 2002;113:1–6.
- Bhatt DL, Marso SP, Lincoff AM, Wolski KE, Ellis SG, Topol EJ. Abciximab reduces mortality in diabetics following percutaneous coronary intervention. *J Am Coll Cardiol*. 2000;35:922–8.
- Bhatt DL, Topol EJ. Current role of platelet glycoprotein IIb/IIIa inhibitors in acute coronary syndromes. *JAMA*. 2000;284:1549–58.
- Montalescot G, Barragan P, Wittenberg O, et al. Platelet glycoprotein IIb/IIIa inhibition with coronary stenting for acute myocardial infarction. *N Engl J Med*. 2001;344:1895–903.
- De Luca G, Suryapranata H, Stone GW, et al. Abciximab as adjunctive therapy to reperfusion in acute ST-segment elevation myocardial infarction: A meta-analysis of randomized trials. *JAMA*. 2005;293:1759–65.
- Topol EJ, Easton D, Harrington RA, et al. Randomized, double-blind, placebo-controlled, international trial of the oral IIb/IIIa antagonist tirofiban in coronary and cerebrovascular disease. *Circulation*. 2003;108:399–406.
- Aktas B, Utz A, Hoenig-Liedl P, Walter U, Geiger J. Dipyridamole enhances NO/cGMP-mediated vasodilator-stimulated phosphoprotein phosphorylation and signaling in human platelets: In vitro and in vivo/ex vivo studies. *Stroke*. 2003;34:764–9.
- Persantine and aspirin in coronary heart disease. The Persantine-Aspirin Reinfarction Study Research Group. *Circulation*. 1980;62:449–61.
- The Persantine-Aspirin Reinfarction Study (PARIS) Research Group. The Persantine-aspirin reinfarction study. *Circulation*. 1980;62:V85–8.
- Klimt CR, Knatterud GL, Stamler J, Meier P. Persantine-Aspirin Reinfarction Study. Part II. Secondary coronary prevention with persantine and aspirin. *J Am Coll Cardiol*. 1986;7:251–69.
- Halkes PH, van Gijn J, Kappelle LJ, Koudstaal PJ, Algra A. Aspirin plus dipyridamole versus aspirin alone after cerebral ischaemia of arterial origin (ESPRIT): Randomised controlled trial. *Lancet*. 2006;367:1665–73.
- Tsuchikane E, Fukuhara A, Kobayashi T, et al. Impact of cilostazol on restenosis after percutaneous coronary balloon angioplasty. *Circulation*. 1999;100:21–6.
- Douglas JS Jr, Holmes DR Jr, Kereiakes DJ, et al. Coronary stent restenosis in patients treated with cilostazol. *Circulation*. 2005;112:2826–32.
- Onoda K, Ohashi K, Hashimoto A, et al. Inhibition of platelet aggregation by combined therapy with aspirin and cilostazol after off-pump coronary artery bypass surgery. *Ann Thorac Cardiovasc Surg*. 2008;14:230–7.
- Kahn ML, Nakanishi-Matsui M, Shapiro MJ, Ishihara H, Coughlin SR. Protease-activated receptors 1 and 4 mediate activation of human platelets by thrombin. *J Clin Invest*. 1999;103:879–87.

31. Wu CC, Teng CM. Comparison of the effects of PAR1 antagonists, PAR4 antagonists, and their combinations on thrombin-induced human platelet activation. *Eur J Pharmacol.* 2006;546:142–7.
32. Chackalamanni S, Wang Y, Greenlee WJ, et al. Discovery of a novel, orally active himbacine-based thrombin receptor antagonist (SCH 530348) with potent antiplatelet activity. *J Med Chem.* 2008;51:3061–4.
33. Lasser G, Guchhait P, Ellsworth JL, et al. C1qTNF-related protein-1 (CTRP-1): A vascular wall protein that inhibits collagen-induced platelet aggregation by blocking VWF binding to collagen. *Blood.* 2006;107:423–30.
34. Bigalke B, Geisler T, Stellos K, et al. Platelet collagen receptor glycoprotein VI as a possible novel indicator for the acute coronary syndrome. *Am Heart J.* 2008;156:193–200.
35. Meier TR, Myers DD Jr, Wroblewski SK, et al. Prophylactic P-selectin inhibition with PSI-421 promotes resolution of venous thrombosis without anticoagulation. *Thromb Haemost.* 2008;99:343–51.
36. Ren YJ, Lu AG, Zhang GQ, Jia DH. Inhibitory effects of nimodipine on platelet aggregation and thrombosis. *Acta Pharmacologica Sinica.* 1998;19:158–60.
37. Hsiao G, Ko FN, Jong TT, Teng CM. Antiplatelet action of 3',4'-diisovalerylhellactone diester purified from *Peucedanum japonicum* Thunb. *Biol Pharm Bull.* 1998;21:688–92.
38. Goldman S, Copeland J, Moritz T, et al. Internal mammary artery and saphenous vein graft patency. Effects of aspirin. *Circulation.* 1990;82:IV237–42.
39. Goldman S, Copeland J, Moritz T, et al. Long-term graft patency (3 years) after coronary artery surgery. Effects of aspirin: Results of a VA Cooperative study. *Circulation.* 1994;89:1138–43.
40. Mangano DT. Aspirin and mortality from coronary bypass surgery. *N Engl J Med.* 2002;347:1309–17.
41. Filion KB, Pilote L, Rahme E, Eisenberg MJ. Use of perioperative cardiac medical therapy among patients undergoing coronary artery bypass graft surgery. *J Card Surg.* 2008;23:209–15.
42. Fitzgibbon GM, Kafka HP, Leach AJ, Keon WJ, Hooper GD, Burton JR. Coronary bypass graft fate and patient outcome: Angiographic follow-up of 5,065 grafts related to survival and reoperation in 1,388 patients during 25 years. *J Am Coll Cardiol.* 1996;28:616–26.
43. Antiplatelet Trialists' Collaboration. Collaborative overview of randomised trials of antiplatelet therapy—II: Maintenance of vascular graft or arterial patency by antiplatelet therapy. *BMJ.* 1994;308:159–68.
44. Antiplatelet Trialists' Collaboration. Collaborative overview of randomised trials of antiplatelet therapy—I: Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. *BMJ.* 1994;308:81–106.
45. Antiplatelet Trialists' Collaboration. Collaborative overview of randomised trials of antiplatelet therapy—III: Reduction in venous thrombosis and pulmonary embolism by antiplatelet prophylaxis among surgical and medical patients. *BMJ.* 1994;308:235–46.
46. Ferraris VA, Ferraris SP, Joseph O, Wehner P, Mentzer RM Jr. Aspirin and postoperative bleeding after coronary artery bypass grafting. *Ann Surg.* 2002;235:820–7.
47. Hongo RH, Ley J, Dick SE, Yee RR. The effect of clopidogrel in combination with aspirin when given before coronary artery bypass grafting. *J Am Coll Cardiol.* 2002;40:231–7.
48. Chu MW, Wilson SR, Novick RJ, Stitt LW, Quantz MA. Does clopidogrel increase blood loss following coronary artery bypass surgery? *Ann Thorac Surg.* 2004;78:1536–41.
49. Kamran M, Ahmed A, Dar MI, Khan AB. Effect of aspirin on postoperative bleeding in coronary artery bypass grafting. *Ann Thorac Cardiovasc Surg.* 2008;14:224–9.
50. Sun JC, Whitlock R, Cheng J, et al. The effect of pre-operative aspirin on bleeding, transfusion, myocardial infarction, and mortality in coronary artery bypass surgery: A systematic review of randomized and observational studies. *Eur Heart J.* 2008;29:1057–71.
51. Dacey LJ, Munoz JJ, Johnson ER, et al. Effect of preoperative aspirin use on mortality in coronary artery bypass grafting patients. *Ann Thorac Surg.* 2000;70:1986–90.
52. Leong JY, Baker RA, Shah PJ, Cherian VK, Knight JL. Clopidogrel and bleeding after coronary artery bypass graft surgery. *Ann Thorac Surg.* 2005;80:928–33.
53. Ouattara A, Bouzguenda H, Le Manach Y, et al. Impact of aspirin with or without clopidogrel on postoperative bleeding and blood transfusion in coronary surgical patients treated prophylactically with a low-dose of aprotinin. *Eur Heart J.* 2007;28:1025–32.
54. Bednar F, Osmancik P, Hlavicka J, Jedlickova V, Paluch Z, Vanek T. Aspirin is insufficient in inhibition of platelet aggregation and thromboxane formation early after coronary artery bypass surgery. *J Thromb Thrombolysis.* 2008;27:394–9.
55. Chen L, Bracey AW, Radovancevic R, et al. Clopidogrel and bleeding in patients undergoing elective coronary artery bypass grafting. *J Thorac Cardiovasc Surg.* 2004;128:425–31.
56. van der Linden J, Lindvall G, Sartipy U. Aprotinin decreases postoperative bleeding and number of transfusions in patients on clopidogrel undergoing coronary artery bypass graft surgery: A double-blind, placebo-controlled, randomized clinical trial. *Circulation.* 2005;112:1276–80.
57. Sirs JA, Glenville B, Stanbridge RL, et al. The influence of vascular pressure and different primes on the fluid balance of, and oxygen supply to, the patient during CABG. *Perfusion.* 1995;10:33–44.
58. Li S, Price R, Phiroz D, Swan K, Crane TA. Systemic inflammatory response during cardiopulmonary bypass and strategies. *J Extra Corpor Technol.* 2005;37:180–8.
59. Farneti PA, Sbrana S, Spiller D, et al. Reduction of blood coagulation and monocyte-platelet interaction following the use of a minimal extracorporeal circulation system (Syner) in coronary artery bypass grafting (CABG). *Perfusion.* 2008;23:49–56.
60. Clar A, Bowers MC, Larson DF. Derivation of sieving coefficients to determine the efficacy of the hemoconcentrator in removal of four inflammatory mediators produced during cardiopulmonary bypass. *ASAIO J.* 1997;43:163–70.
61. Chen YF, Tsai WC, Lin CC, et al. Effect of leukocyte depletion on endothelial cell activation and transendothelial migration of leukocytes during cardiopulmonary bypass. *Ann Thorac Surg.* 2004; 78: 634–42; discussion 642–33.
62. Sobieski MA II, Graham JD, Pappas PS, Tatoes AJ, Slaughter MS. Reducing the effects of the systemic inflammatory response to cardiopulmonary bypass: Can single dose steroids blunt systemic inflammatory response syndrome? *ASAIO J.* 2008;54:203–6.
63. Elzey BD, Sprague DL, Ratliff TL. The emerging role of platelets in adaptive immunity. *Cell Immunol.* 2005;238:1–9.
64. Henn V, Slupsky JR, Grafe M, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature.* 1998;391:591–4.
65. Henn V, Steinbach S, Buchner K, Presek P, Kroccek RA. The inflammatory action of CD40 ligand (CD154) expressed on activated human platelets is temporally limited by coexpressed CD40. *Blood.* 2001;98:1047–54.
66. Lee Y, Lee WH, Lee SC, et al. CD40L activation in circulating platelets in patients with acute coronary syndrome. *Cardiology.* 1999;92:11–6.
67. Nannizzi-Alaimo L, Alves VL, Phillips DR. Inhibitory effects of glycoprotein IIb/IIIa antagonists and aspirin on the release of soluble CD40 ligand during platelet stimulation. *Circulation.* 2003;107:1123–8.
68. Azar RR, Kassab R, Zoghbi A, et al. Effects of clopidogrel on soluble CD40 ligand and on high-sensitivity C-reactive protein in patients with stable coronary artery disease. *Am Heart J.* 2006;151:521.e1–521.e4.
69. Nannizzi-Alaimo L, Rubenstein MH, Alves VL, Leong GY, Phillips DR, Gold HK. Cardiopulmonary bypass induces release of soluble CD40 ligand. *Circulation.* 2002;105:2849–54.
70. Steinhubl SR, Talley JD, Braden GA, et al. Point-of-care measured platelet inhibition correlates with a reduced risk of an adverse cardiac event after percutaneous coronary intervention: Results of the GOLD (AU-Assessing Ultegra) multicenter study. *Circulation.* 2001;103:2572–8.
71. Gum PA, Kottke-Marchant K, Poggio ED, et al. Profile and prevalence of aspirin resistance in patients with cardiovascular disease. *Am J Cardiol.* 2001;88:230–5.
72. Macchi L, Christiaens L, Brabant S, et al. Resistance in vitro to low-dose aspirin is associated with platelet PIA1 (GP IIIa) polymorphism but not with C807T (GP Ia/IIa) and C-5T Kozak (GP Ibalpha) polymorphisms. *J Am Coll Cardiol.* 2003;42:1115–9.
73. Lev EI, Patel RT, Maresh KJ, et al. Aspirin and clopidogrel drug response in patients undergoing percutaneous coronary intervention: The role of dual drug resistance. *J Am Coll Cardiol.* 2006;47:27–33.
74. Cattaneo M. Aspirin and clopidogrel: Efficacy, safety, and the issue of drug resistance. *Arterioscler Thromb Vasc Biol.* 2004;24:1980–7.

75. Catella-Lawson F, Reilly MP, Kapoor SC, et al. Cyclooxygenase inhibitors and the antiplatelet effects of aspirin. *N Engl J Med.* 2001;345:1809–17.
76. Lau WC, Waskell LA, Watkins PB, et al. Atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation: A new drug-drug interaction. *Circulation.* 2003;107:32–7.
77. Borgdorff P, Tangelder GJ, Paulus WJ. Cyclooxygenase-2 inhibitors enhance shear stress-induced platelet aggregation. *J Am Coll Cardiol.* 2006;48:817–23.
78. Halushka MK, Halushka PV. Why are some individuals resistant to the cardioprotective effects of aspirin? Could it be thromboxane A₂? *Circulation.* 2002;105:1620–2.
79. Feher G, Feher A, Pusch G, Lupkovic G, Szapary L, Papp E. The genetics of antiplatelet drug resistance. *Clin Genet.* 2009;75:1–18.
80. Hobson AR, Agarwala RA, Swallow RA, Dawkins KD, Curzen NP. Thrombelastography: Current clinical applications and its potential role in interventional cardiology. *Platelets.* 2006;17:509–18.
81. Tantry US, Bliden KP, Gurbel PA. Overestimation of platelet aspirin resistance detection by thrombelastograph platelet mapping and validation by conventional aggregometry using arachidonic acid stimulation. *J Am Coll Cardiol.* 2005;46:1705–9.
82. Bochsén L, Wiinberg B, Kjelgaard-Hansen M, Steinbruchel DA, Johansson PI. Evaluation of the TEG platelet mapping assay in blood donors. *Thromb J.* 2007;5:3.
83. Alstrom U, Tyden H, Oldgren J, Siegbahn A, Stahle E. The platelet inhibiting effect of a clopidogrel bolus dose in patients on long-term acetylsalicylic acid treatment. *Thromb Res.* 2007;120:353–9.
84. Syrbe G, Redlich H, Weidlich B, et al. Individual dosing of ASA prophylaxis by controlling platelet aggregation. *Clin Appl Thromb Hemost.* 2001;7:209–13.
85. Helgason CM, Bolin KM, Hoff JA, et al. Development of aspirin resistance in persons with previous ischemic stroke. *Stroke.* 1994;25:2331–6.
86. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *BMJ.* 2002;324:71–86.
87. Serebruany VL, Malinin AI, Sane DC, et al. Magnitude and time course of platelet inhibition with Aggrenox and aspirin in patients after ischemic stroke: The AGgrenox versus Aspirin Therapy Evaluation (AGATE) trial. *Eur J Pharmacol.* 2004;499:315–24.
88. Michelson AD, Cattaneo M, Eikelboom JW, et al. Aspirin resistance: Position paper of the Working Group on Aspirin Resistance. *J Thromb Haemost.* 2005;3:1309–11.
89. Bedard PW, Clerin V, Sushkova N, et al. Characterization of the novel P-selectin inhibitor PSI-697 [2-(4-chlorobenzyl)-3-hydroxy-7,8,9,10-tetrahydrobenzo[h]quinoline-4-carboxylic acid] in vitro and in rodent models of vascular inflammation and thrombosis. *J Pharmacol Exp Ther.* 2008;324:497–506.