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Review Article

Thienopyridines, Beyond ADP Antagonism: A Review of Pleiotropic Effects

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Abstract

Platelet activation is fundamentally involved in the initiation and development of atherosclerotic disease. It orchestrates the triad of inflammation, atherosclerosis and thrombosis. Thienopyrindines are one of the most commonly prescribed drugs for secondary prevention of cardiovascular diseases. Their mode of action is through irreversible inhibition of $P2Y_{12}$ receptors on platelets and thereby antagonizing the effect of adenosine diphosphate (ADP) resulting in potent anti-platelet effects. There are, however, emerging data over last decade, which indirectly support ADP-independent effects of thienopyridine drugs with multiple beneficial modes of action in cardiovascular disease prevention. In this review, we focus on the potential pleiotropic effects of thienopyridines.

Introduction

Until the last few years, platelets were considered to be an anuclear, disc-shaped cell involved in hemostasis and thrombus formation, a normal physiological response of the vasculature to an injury causing bleeding. However, when platelet activation and aggregation occur abnormally in the intact vasculature, this results in acute coronary syndrome (ACS), stroke, or end-organ ischemia and infarction. The thienopyridine anti-platelet agents, clopidogrel and prasugrel (ticlopidine is no longer commonly used due to its side effect profile) have become the cornerstone therapy for secondary prevention of ischemic cardiovascular events with numerous large-scale outcome driven randomized studies demonstrating their efficacy in patients with coronary artery disease [1-3].

Thienopyridines are pro-drugs that require biotransformation into their active metabolites. Differences in individual drug metabolism arise from polymorphisms of genes involved with biotransformation and/or metabolism of thienopyridines. Polymorphism involving CYP2C19 gene is the most commonly studied variations associated with reduced clopidogrel effectiveness.

Observational studies suggest that genetic polymorphisms resulting in poor metabolism are associated with adverse cardiovascular outcomes [4], but randomized studies have failed to show any such differences in clinical outcomes between patients with different polymorphisms [5,6]. Similarly, studies of rapid metabolizers have also failed to demonstrate any beneficial effect on stent thrombosis despite marked inhibition to ADP induced platelet aggregation, and excessive bleeding risk [7].

Discrepancies between laboratory based experimental data, observational studies and randomized controlled trials of clinical endpoints, suggest that the thienopyridine group of medications not only inhibits platelet activation and aggregation through irreversible blockade of $P2Y_{12}$ receptor by disrupting homoligomers of $P2Y_{12}$ receptors into non-functional dimers and monomers [8], but also may exhibit other important mechanisms of actions independent of the ADP receptor. These actions include modulation of immunity, inflammation, and redox milieu, altering platelet-leucocyte-monocyte interactions as well as initiation and progression of atherosclerosis [9-11]. In this review article, we highlight potential additional mechanisms of actions of thienopyridines pertaining to their role in cardiovascular disease.

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Anti-platelet effects in addition to anti-ADP mechanism

Beyond inhibiting ADP receptors, clopidogrel can also reduce platelet thromboxane A2 (TXA2) production [12], and reduce arachidonic acid (AA)-induced platelet aggregation, irrespective of TXA2 level [13] suggesting that clopidogrel may also potentiate aspirin's platelet inhibitory effect. In support of this, experimental studies have shown that upon clopidogrel withdrawal, there is incremental increase in AA-induced platelet activation, which is independent of aspirin effect [14], suggesting that clopidogrel exhibits additional anti-platelet action through 'aspirin specific pathway' [15].

One of the most potent platelet agonists in vivo is collagen [16], especially in presence of dysfunctional or denuded endothelium [17]. Clopidogrel can inhibit collagen-induced platelet aggregation in whole blood (less potent than its anti-ADP effect) as well as platelet-subendothelial interaction under-flowing condition [16]. Intra platelet phosphoinositol-TXA2 pathway is involved in collagen induced platelet aggregation, suggesting that there is significant cross talk between various pathways leading to platelet aggregation.

In contrast to the general belief that thienopyridines do not act on protease-activated receptor-1 (PAR-1), a receptor for thrombin, the most potent platelet agonist known yet, there are reports of clopidogrel significantly inhibiting Thrombin Receptor Activating Peptide (TRAP) induced platelet aggregation and procoagulant activity in patients with coronary artery disease [18,19]. Additionally clopidogrel withdrawal after 1 year in patients who have undergone percutaneous coronary intervention (PCI) is associated with an increment in thrombin induced platelet aggregation [20], whereas no such results were seen in healthy volunteers after short course of clopidogrel, [21] suggesting that these additional beneficial effect of clopidogrel may be observed only in those with activated platelets, but not in cohort of healthy subjects.

Prostacyclin, an eicosanoid with vaso-dilatatory and anti-platelet aggregatory properties is mainly released from endothelial cells. Aspirin inhibits prostacyclin production in a dose-dependent manner [22,23], whereas thienopyridines do not appear to have such inhibitory effects. However, thienopyridines and their R-enantiomers, devoid of any anti-platelet actions, can stimulate prostacyclin (PGI2) and tissue plasminogen activator (t-PA) production resulting in thrombolysis much earlier than their anti-platelet actions are exerted [24,25]. Additionally, prostacyclin inhibits thrombin induced platelet aggregation only in presence of clopidogrel, whereas no such effect was observed in the absence of $P2Y_{12}$ receptor inhibition [26].

Epinephrine is a platelet agonist, which can potentiate the effects of other agonists on platelet aggregation and activation [27,28]. Clopidogrel withdrawal has been associated with significant increase in epinephrine-induced platelet aggregation [20]. It remains to be elucidated how clopidogrel impairs epinephrine-induced platelet aggregation as the down-stream pathway for epinephrine stimulated α2 receptor (Gz coupled receptor) activation is different than the $P2Y_{12}$ receptor activation (Gi coupled receptor). Nevertheless, these findings do suggest that there are various pathways leading to platelet activation, which are intimately linked to each other and that thienopyridines might exert significant inhibitory effect on other receptor mediated platelet activation beyond $P2Y_{12}$ inhibition [29], resulting in overall potent anti-platelet effect. Most of the studies evaluating clopidogrel or thienopyridine efficacy on platelet inhibition have not evaluated $P2Y_{12}$ independent mechanisms.

Effects on endothelial and vasculature function

In addition to known thrombotic activity, platelets are involved in endothelial cell activation as well as initiation and progression of atherosclerosis [30-33]. Platelet activation and activity are reciprocally affected by the endothelium, suggesting endothelial-platelet functional coupling [34,35]. For example, bacteria-activated platelets have been shown to induce endothelial cell apoptosis via capase-8 & 9 and generation of reactive oxygen species (ROS) [36].

Studies more than a decade ago suggested that thienopyridines might have important vasomodulatory actions. In animal studies, intravenous clopidogrel and ticlopidine markedly reduced vasoconstriction in response to srotonin, endothelin-1, serum and platelet rich plasma/arachidonic acid mixture [37,38]. A single loading dose of clopidogrel causes dose-dependent endothelial function improvement [39]. Thienopyridines and their R-enantiomer (free of any anti-platelet actions) can stimulate nitric oxide (NO) release from endothelial cells [40] in a dose-dependent manner [16] in addition to prostacyclin and tissue plasminogen activator (t-PA)(24,25), as described earlier. The underlying mechanism leading to increase NO bioavailability could be interleukin 1β-stimulated NO release [41], an action that is through non-purinoceptors related pathway [42].

Studies of glycoprotein IIb/IIIa receptor blockade report improvement in platelet and endothelial NO bioavailability [43,44]. Thienopyridines are known to inhibit platelet IIb/IIIa expression, a marker of platelet activation [45]. Clopidogrel therapy, therefore, has been associated with improvement in vascular endothelial function [9,46].

Circulating endothelial cell (CEC) is a marker of endothelial dysfunction, an underlying basic mechanism eventually leading to coronary artery disease (CAD) [47]. CEC is positively associated with not only the extent of the disease, but also

characterized by a rise in CEC, was markedly inhibited in patients who had higher platelet inhibition with clopidogrel than those who still had high on-treatment platelet reactivity [49], suggesting a vasoprotective role of thienopyridines.

In context of current data, the effect of clopidogrel on the vasculature is likely multiple with indirect effects through modulation of platelet function/release of chemokines, and/or direct effect on vascular endothelial cell $P2Y_{12}$ receptors. Recently $P2Y_{12}$ receptors have been identified on rat brain capillary endothelial cells [50], human coronary arterial endothelial cells, human umbilical vein endothelial cells (HUVEC) as well as human aortic smooth muscle cells [51]. A study in $P2Y_{12}$ deficient mice suggested that platelet $P2Y_{12}$ receptors have more impact than vascular endothelial $P2Y_{12}$ receptor on injury-induced vascular endothelial response and thrombosis [52]. Thienopyridines can form nitrosothiol derivatives (thienopyridine-SNO) in the presence of nitrite (available in circulation), without the need for drug metabolism [53]. Increased availability of nitrate resulted in incremental thienopyridine-SNO formation that can induce endothelium-independent vasodilation, in a pH-dependent manner. There are intra-group differences in thienopyridine-SNO formation and the resultant increase in NO bioavailability partly explains differences in their effect on vascular endothelial and platelet function [53,54].

Effects on redox signaling

Platelets are an important source of ROS in the vasculature [55-58]. ROS very quickly reacts with available NO to form peroxynitrite diminishing the bioavailability of NO. Decreased NO bioavailability is characteristic of patients with risk factors for cardiovascular disease, e.g., hypertension, smoker, diabetes mellitus and hypercholesterolemia, as well as in patients with established coronary artery disease and heart failure. Platelets harbor multiple sources of ROS including nicotinamide adenine dinucleotide (phosphate) NADP/NADPH oxidase, cyclooxygenase, uncoupled nitric oxide synthase (NOS) and xanthine oxidase (XO). Neutrophils are a very potent source of ROS in comparison to platelets. Platelets not only release ROS themselves, but also stimulate neutrophils to generate ROS, which in turn can activate platelets further in a positive loop fashion [59]. Clopidogrel treatment has been associated with marked reduction in platelet P-selectin expression and ROS production from neutrophils in animal models and human subjects [60]. Similarly, recombinant soluble CD40 Ligand (CD40L) can stimulate ROS generation from vascular cells while clopidogrel therapy resulted in CD40L suppression suggesting overall beneficial effect of clopidogrel on the redox milieu [61]. Additionally, clopidogrel was shown to reduce ROS production in patients with CAD whereas aspirin did not demonstrate this beneficial effect [9].

Effects on platelet-leukocyte interaction

Platelet–white blood cell (neutrophil, monocyte, and phocyte) interaction has emerged as a vital underlying regulatory mechanism leading to inflammation, atherosclerosis and thrombosis [62]. Platelets upon activation express P-selectin from secretory α-granule on to their membranes, which can crosslink with its receptor P-selectin glycoprotein ligand-1 (PSGL-1), present on cells such as the endothelium where it helps platelets to tether, roll and eventually firmly adhere. Similar binding also occurs with monocytes, neutrophils and lymphocytes [63,64], that increases leucocyte adhesiveness and help them roll and adhere on surface-arrested platelets [65]. This process involves up-regulation of β1 and β2 integrin [66], followed by diapedesis and migration of these cells across the endothelium.

Similar to leukocyte interaction, platelets help lymphocytes migrate between blood and lymphatic channels gain using P-selectin-PSGL-1 interaction. Activated platelets also secrete Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES) platelet microparticles, which deposit on activated endothelium and enhance monocyte recruitment, a process that can be inhibited by blocking GPIb, GPIIb/IIIa, P-selectin and junctional adhesion molecule-A [67]. CD40L secreted from platelets attaches to its receptor on various cells involved in atherosclerosis, plaque instability and thrombosis [68]. Stimulated platelets not only activate leukocytes but can also initiate chemokine synthesis [69] and induction of cytokine expression [70].

Clopidogrel therapy has been associated with significant reduction in spontaneous platelet-monocyte and platelet-neutrophil conjugates as well as plasma levels of P-selectin and CD40L in patients with ACS [19]. In animal models of transplant arteriosclerosis, clopidogrel therapy resulted in significantly reduced blood levels of CD40L and P-selectin, cellular level of P-selectin, E-selectin, intracellular adhesion molecule (ICAM) and platelet derived growth factor β (PDGF) expression, confirmed by intra-graft mRNA [71]. Prasugrel, another thienopyridine, was shown to be more potent than clopidogrel in attenuating platelet monocyte aggregates [45]. Therefore, platelets appear to have a role as a key mediator of systemic vascular inflammation by attracting white cells towards sites of dysfunctional endothelium, in a very complex, but well controlled manner.

Effects on immunity

Similar to various risk factors and CAD itself, sepsis induces platelet activation resulting in micro-angiopathy, disseminated intravascular coagulation and probably modifying inflammatory responses leading to multi-organ failure [72]. Thrombocytopenia is considered to be a poor prognostic marker in septic patients [73].

Patients admitted to intensive care with pneumonia, who were treated with aspirin or clopidogrel, had shorter in-hospital stay, despite being nearly 12 years older than their controls [74]. Similarly in the animal studies

beneficial effects of clopidogrel therapy were observed in a mouse model of polymicrobial sepsis [75]. Clopidogrel inhibited endotoxin-induced up-regulation of inflammatory related genes. The use of antiplatelet therapy is also associated with reduced organ failure and improved mortality in general intensive care patients [76].

In a transgenic mouse model of chronic hepatitis B, combined anti-platelet medications including aspirin and clopidogrel, rather than a single agent alone significantly improved mortality without increasing bleeding. Proposed mechanism resulting in mortality benefit was thought to be the inhibitory effect on accumulation of virus specific CD8+ T cell as well as generalized inflammatory response. In these animal models inhibited hepatocyte proliferation as well as liver fibrosis was also observed. Anti-platelet therapy, however, failed to demonstrate similar beneficial effect on chemical induced liver carcinogenesis, suggesting that the underlying beneficial mechanism is related immune and inflammatory response modulation [77].

Effects on inflammation and atherosclerosis

Atherosclerosis is considered to be fundamentally an inflammatory process [78]. Interestingly, evidences suggest that several established therapies that primarily treat coronary risk factors such as hypertension, diabetes ellitus and dyslipidemia, may have inhibitory effect on inflammatory markers [79-81], suggesting a key role of inflammation in the initiation and progression of atherosclerosis.

C-reactive protein (CRP) is an inflammatory marker associated with underlying coronary artery disease and complications of atherothrombosis [82]. In patients undergoing PCI, increased CRP post stenting was associated with excessive restenosis post-procedure [83]. Clopidogrel use was associated with suppression of CRP values in a time-dependent manner [84]. Further evidence of an indirect effect of clopidogrel on inflammation comes from studies reporting an association between clopidogrel treatment and markedly reduced levels of CD40L release [85,86], hsCRP, P-selectin expression and platelet-leukocyte aggregates [87]. Similarly, prasugrel has been observed to be more potent in inhibiting surface expression of platelet endothelial cell adhesion molecule (PECAM-1) and GPIIb/IIIa antigen and activity of GPIb, P-selectin, and CD40L than clopidogrel [88]. Even 4 weeks after stopping clopidogrel therapy, hsCRP and interleukin remained persistently low implying that even though platelet aggregation had returned to baseline much earlier, there was additional anti-inflammatory effects of clopidogrel, which persisted for longer [14].

Summary

Platelets are critically involved in the triad of inflammation, atherosclerosis and thrombosis. There are emerging evidence to support beneficial effects of thienopyridines beyond $P2Y_{12}$ receptor blockade and inhibition of ADP-induced platelet aggregation. These effects include increasing NO bioavailability, suppression of inflammatory markers and, perhaps, altering atherosclerosis progression. Despite these plausible mechanisms of action, data supporting their role in primary prevention of cardiovascular disease is lacking. Large prospective trials to examine overall efficacy of thienopyridines in primary prevention and long-term clinical outcomes are warranted.

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Conflict of interest

None

References:

1. Mehta S R, Yusuf S, Clopidogrel in Unstable angina to prevent Recurrent Events Study I. The Clopidogrel in Unstable angina to prevent Recurrent Events (CURE) trial programme; rationale, design and baseline characteristics including a meta-analysis of the effects of thienopyridines in vascular disease. European heart journal 2000, 2: 2033-2041.

2. Hirsh J, Bhatt D L. Comparative benefits of clopidogrel and aspirin in high-risk patient populations: lessons from the CAPRIE and CURE studies. Archives of internal medicine. 2004, 164: 2106-2110.

3. Wiviott S D, Antman E M, Gibson C M, Montalescot G, Riesmeyer J et al. Evaluation of prasugrel compared with clopidogrel in patients with acute coronary syndromes: design and rationale for the TRial to assess Improvement in Therapeutic Outcomes by optimizing platelet InhibitioN with prasugrel Thrombolysis In Myocardial Infarction 38 (TRITON-TIMI 38). American heart journal. 2006, 152: 627-635.

4. Mega J L, Simon T, Collet J P, Anderson J L, Antman E M et al. Reduced-function CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI: a meta-analysis. JAMA : the journal of the American Medical Association. 2010, 304: 1821-1830.

5. Paré G, Mehta S R, Yusuf S, Anand S S, Connolly S J et al. Effects of CYP2C19 genotype on outcomes of clopidogrel treatment. The New England journal of medicine. 2010, 363: 1704-1714.

6. Pare G, Eikelboom J W, Sibbing D, Bernlochner I, Kastrati A. Testing should not be done in all patients treated with clopidogrel who are undergoing percutaneous coronary intervention. Circulation Cardiovascular interventions. 2011, 4: 514-521.

7. Sibbing D, Koch W, Gebhard D, Schuster T, Braun S D et al. Cytochrome 2C19*17 allelic variant, platelet aggregation, bleeding events, and stent thrombosis in clopidogrel-treated patients with coronary stent placement. Circulation. 2010, 121: 512-518.

8. Savi P, Zachayus J L, Delesque-Touchard N, Labouret C, Hervé C et al. The active metabolite of Clopidogrel disrupts P2Y12 receptor oligomers and partitions them out of lipid rafts. Proc Natl Acad Sci U S A. 2006 103: 11069-11074.

9. Heitzer T, Rudolph V, Schwedhelm E, Karstens M, Sydow K et al. Clopidogrel improves systemic endothelial nitric oxide bioavailability in patients with coronary artery disease: evidence for antioxidant and antiinflammatory effects. Arteriosclerosis, thrombosis, and vascular biology. 2006, 26: 1648-1652.

10. Li M, Zhang Y, Ren H, Zhang Y, Zhu X. Effect of clopidogrel on the inflammatory progression of early atherosclerosis in rabbits model. Atherosclerosis. 2007, 194: 348-356.

11. Langer H F, Gawaz M. Platelet-vessel wall interactions in atherosclerotic disease. Thrombosis and haemostasis. 2008, 99: 480-486.

12. Armstrong P C, Dhanji A R, Tucker A T, Mitchell J A, Warner T D. Reduction of platelet thromboxane A2 production *ex vivo* and *in vivo* by clopidogrel therapy. Journal of thrombosis and haemostasis : JTH. 2010, 8: 613-615.

13. Frelinger A L 3rd, Furman M I, Linden M D, Li Y, Fox M L et al. Residual arachidonic acid-induced platelet activation via an adenosine diphosphate-dependent but cyclooxygenase-1- and cyclooxygenase-2-independent pathway: a 700-patient study of aspirin resistance. Circulation. 2006, 113: 2888-2896.

14. Sambu N, Dent H, Englyst N, Warner T D, Leadbeater P et al. Effect of clopidogrel withdrawal on platelet reactivity and vascular inflammatory biomarkers 1 year after drug-eluting stent implantation: results of the prospective, single-centre CESSATION study. Heart. 2011, 97: 1661-1667.

15. Hobson A R, Qureshi Z, Banks P, Curzen N P. Effects of clopidogrel on "aspirin specific" pathways of platelet inhibition. Platelets. 2009, 20: 386-390.

16. Arrebola M M, De la Cruz J P, Villalobos M A, Pinacho A, Guerrero A et al. In vitro effects of clopidogrel on the platelet-subendothelium interaction, platelet thromboxane and endothelial prostacyclin production, and nitric oxide synthesis. Journal of cardiovascular pharmacology. 2004, 43: 74-82.

17. Nieswandt B, Watson S P. Platelet-collagen interaction: is GPVI the central receptor? Blood. 2003, 102: 449-461.

18. Behan M W, Fox S C, Heptinstall S, Storey R F. Inhibitory effects of P2Y12 receptor antagonists on TRAP-induced platelet aggregation, procoagulant activity, microparticle formation and intracellular calcium responses in patients with acute coronary syndromes. Platelets. 2005, 16: 73-80.

19. Xiao Z, Théroux P. Clopidogrel inhibits platelet-leukocyte interactions and thrombin receptor agonist peptide-induced platelet activation in patients with an acute coronary syndrome. Journal of the American College of Cardiology. 2004, 43: 1982-1988.

20. Mylotte D, Peace A J, Tedesco A T et al. Clopidogrel discontinuation and platelet reactivity following coronary stenting. Journal of thrombosis and haemostasis : JTH. 2011, 9: 24-32.

21. Frelinger A L, 3rd, Barnard M R, Fox M L, Michelson A D. The Platelet Activity After Clopidogrel Termination (PACT) study. Circulation Cardiovascular interventions. 2010, 3: 442-449.

22. Masotti G, Galanti G, Poggesi L, Abbate R, Neri Serneri G G. Differential inhibition of prostacyclin production and platelet aggregation by aspirin. Lancet. 1979, 2:v1213-v1217.

23. Czervionke R L, Smith J B, Fry G L, Hoak J C, Haycraft D L. Inhibition of prostacyclin by treatment of endothelium with aspirin. Correlation with platelet adherence. The Journal of clinical investigation. 1979, 63: 1089-1092.

24. Gryglewski R J, Dupin J P, Uracz W, Swiês J, Madej J et al. Thrombolysis by thienopyridines and their congeners. J Physiol Pharmacol. 2000, 51: 683-693.

25. Gryglewski R J, Korbut R, Swies J, Kostka Trabka E et al. Thrombolytic action of ticlopidine: possible mechanisms. Eur J Pharmacol. 1996, 308: 61-67.

26. Cattaneo M, Lecchi A. Inhibition of the platelet P2Y12 receptor for adenosine diphosphate potentiates the antiplatelet effect of prostacyclin. Journal of thrombosis and haemostasis : JTH. 2007, 5: 577-582.

27. Anfossi G, Trovati M. Role of catecholamines in platelet function: pathophysiological and clinical significance. European journal of clinical investigation. 1996, 26: 353-370.

28. Maayani S, Schwarz T, Craddock-Royal B, Tagliente TM. Activation of the alpha(2A)-adrenoceptor mediates deceleration of the deaggregation component of the response to ADP or 5-HT in human platelets in vitro. Platelets. 2001, 12: 359-375.

29. Storey R F, Sanderson H M, White A E, May J A, Cameron KE et al. The central role of the P(2T) receptor in amplification of human platelet activation, aggregation, secretion and procoagulant activity. British journal of haematology. 2000, 110: 925-934.

30. Schober A, Manka D, von Hundelshausen P, Huo Y, Hanrath P et al. Deposition of platelet RANTES triggering monocyte recruitment requires P-selectin and is involved in neointima formation after arterial injury. Circulation. 2002, 106: 1523-1529.

31. Henn V, Slupsky J R, Gräfe M, Anagnostopoulos I, Förster R et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. Nature. 1998, 391: 591-594.

32. Massberg S, Brand K, Grüner S, Page S, Müller E et al. A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. The Journal of experimental medicine. 2002, 196: 887-896.

33. Weber C. Platelets and chemokines in atherosclerosis: partners in crime. Circulation research. 2005, 96: 612-616.

34. Pohl U, Busse R. Endothelium-dependent modulation of vascular tone and platelet function. European heart journal. 1990, 11 Suppl B: 35-42.

35. Malmstrom R E, Settergren M, Pernow J. Endothelin attenuates endothelium-dependent platelet inhibition in man. Acta physiologica. 2010, 198: 441-448.

36. Kuckleburg C J, Tiwari R, Czuprynski C J. Endothelial cell apoptosis induced by bacteria-activated platelets requires caspase-8 and -9 and generation of reactive oxygen species. Thrombosis and haemostasis. 2008, 99: 363-372.

37. Yang L H, Hoppensteadt D, Fareed J. Modulation of vasoconstriction by clopidogrel and ticlopidine. Thrombosis research. 1998, 92: 83-89.

38. Yang L H, Fareed J. Vasomodulatory action of clopidogrel and ticlopidine. Thrombosis research. 1997, 86: 479-491.

39. Warnholtz A, Ostad M A, Velich N, Trautmann C, Schinzel R et al. A single loading dose of clopidogrel causes dose-dependent improvement of endothelial dysfunction in patients with stable coronary artery disease: results of a double-blind, randomized study. Atherosclerosis. 2008, 196: 689-695.

40. Jakubowski A, Chlopicki S, Olszanecki R, Jawien J, Lomnicka M et al. Endothelial action of thienopyridines and thienopyrimidinones in the isolated guinea pig heart. Prostaglandins, leukotrienes, and essential fatty acids. 2005, 72: 139-145.

41. Inoue M, Kusano E, Ito C, Akimoto T, Iimura O et al. The anti-platelet agent, ticlopidine, upregulates interleukin-1-Beta-stimulated nitric oxide production in cultured rat vascular smooth muscle cells. Experimental nephrology. 2002, 10: 267-274.

42. Froldi G, Bertin R, Dorigo P, Montopoli M, Caparrotta L. Endothelium-independent vasorelaxation by ticlopidine and clopidogrel in rat caudal artery. The Journal of pharmacy and pharmacology. 2011, 63: 1056-1062.

43. Heitzer T, Ollmann I, Koke K, Meinertz T, Munzel T. Platelet glycoprotein IIb/IIIa receptor blockade improves vascular nitric oxide bioavailability in patients with coronary artery disease. Circulation. 2003, 108: 536-541.

44. Chakrabarti S, Clutton P, Varghese S, Cox D, Mascelli MA et al. Glycoprotein IIb/IIIa inhibition enhances platelet nitric oxide release. Thrombosis research. 2004, 113: 225-233.

45. Braun O O, Johnell M, Varenhorst C, James S, Brandt J T et al. Greater reduction of platelet activation markers and platelet-monocyte aggregates by prasugrel compared to clopidogrel in stable coronary artery disease. Thrombosis and haemostasis. 2008, 100: 626-633.

46. Schäfer A, Fraccarollo D, Pförtsch S, Loch E, Neuser J et al. Clopidogrel improves endothelial function and NO bioavailability by sensitizing adenylyl cyclase in rats with congestive heart failure. Basic Res Cardiol. 2011, 106: 485-494.

47. Blann A D, Woywodt A, Bertolini F, Bull T M, Buyon J P et al. Circulating endothelial cells. Biomarker of vascular disease. Thrombosis and haemostasis. 2005, 93: 228-235.

48. Sabatier F, Camoin-Jau L, Anfosso F, Sampol J, Dignat-George F. Circulating endothelial cells, microparticles and progenitors: key players towards the definition of vascular competence. Journal of cellular and molecular medicine. 2009, 13: 454-471.

49. Bonello L, Harhouri K, Sabatier F, Camoin-Jau L, Arnaud L et al. Level of adenosine diphosphate receptor P2Y12 blockade during percutaneous coronary intervention predicts the extent of endothelial injury, assessed by circulating endothelial cell measurement. Journal of the American College of Cardiology. 2010, 56: 1024-1031.

50. Simon J, Filippov AK, Göransson S, Wong Y H, Frelin C et al. Characterization and channel coupling of the P2Y(12) nucleotide receptor of brain capillary endothelial cells. The Journal of biological chemistry. 2002, 277: 31390-31400.

51. Shanker G, Kontos J L, Eckman D M, Wesley-Farrington D, Sane D C. Nicotine upregulates the expression of P2Y12 on vascular cells and megakaryoblasts. Journal of thrombosis and thrombolysis. 2006, 22: 213-220.

52. Evans D J, Jackman L E, Chamberlain J, Crosdale D J, Judge H M et al. Platelet P2Y (12) receptor influences the vessel wall response to arterial injury and thrombosis. Circulation. 2009, 119: 116-122.

53. Bundhoo S S, Anderson R A, Sagan E, Hassan N, Pinder A G et al. Direct formation of thienopyridine-derived nitrosothiols--just add nitrite! Eur J Pharmacol. 2011, 670: 534-540.

54. Bundhoo S S, Anderson R A, Sagan E, Dada J, Harris R et al. Direct vasoactive properties of thienopyridine-derived nitrosothiols. Journal of cardiovascular pharmacology. 2011, 58: 550-558.

55. Chakrabarti S, Varghese S, Vitseva O, Tanriverdi K, Freedman J E. CD40 ligand influences platelet release of reactive oxygen intermediates. Arteriosclerosis, thrombosis, and vascular biology. 2005, 25: 2428-2434.

56. Clutton P, Miermont A, Freedman J E. Regulation of endogenous reactive oxygen species in platelets can reverse aggregation. Arteriosclerosis, thrombosis, and vascular biology. 2004, 24: 187-192.

57. Shah A, Passacquale G, Gkaliagkousi E, Ritter J, Ferro A. Platelet nitric oxide signalling in heart failure: role of oxidative stress. Cardiovascular research. 2011, 91: 625-631.

58. Freedman J E. Oxidative stress and platelets. Arteriosclerosis, thrombosis, and vascular biology. 2008, 28: s11-6.

59. Vanichakarn P, Blair P, Wu C, Freedman J E, Chakrabarti S. Neutrophil CD40 enhances platelet-mediated inflammation. Thrombosis research. 2008, 122: 346-358.

60. Evangelista V, Manarini S, Dell'Elba G, Martelli N, Napoleone E et al. Clopidogrel inhibits platelet-leukocyte adhesion and platelet-dependent leukocyte activation. Thrombosis and haemostasis. 2005, 94: 568-577.

61. Chakrabarti S, Rizvi M, Morin K, Garg R, Freedman JE. The role of CD40L and VEGF in the modulation of angiogenesis and inflammation. Vascular pharmacology. 2010, 53: 130-137.

62. Freedman JE, Loscalzo J. Platelet-monocyte aggregates: bridging thrombosis and inflammation. Circulation. 2002, 105: 2130-2132.

63. Larsen E, Celi A, Gilbert G E, Furie B C, Erban J K, et al. PADGEM protein: a receptor that mediates the interaction of activated platelets with neutrophils and monocytes. Cell. 1989, 59: 305-312.

64. von Hundelshausen P, Weber C. Platelets as immune cells: bridging inflammation and cardiovascular disease. Circulation research. 2007, 100: 27-40.

65. Diacovo T G, Roth S J, Buccola J M, Bainton D F, Springer T A. Neutrophil rolling, arrest, and transmigration across activated, surface-adherent platelets via sequential action of P-selectin and the beta 2-integrin CD11b/CD18. Blood. 1996, 88: 146-157.

66. da Costa Martins P A, van Gils J M, Mol A, Hordijk P L, Zwaginga J J. Platelet binding to monocytes increases the adhesive properties of monocytes by up-regulating the expression and functionality of beta1 and beta2 integrins. Journal of leukocyte biology. 2006, 79: 499-507.

67. Mause S F, von Hundelshausen P, Zernecke A, Koenen RR, Weber C. Platelet microparticles: a transcellular delivery system for RANTES promoting monocyte recruitment on endothelium. Arteriosclerosis, thrombosis, and vascular biology. 2005, 25: 1512-1518.

68. Chakrabarti S, Blair P, Freedman J E. CD40-40L signaling in vascular inflammation. The Journal of biological chemistry. 2007, 282: 18307-18317.

69. Weyrich A S, Elstad M R, McEver R P, McIntyre T M, Moore K L et al. Activated platelets signal chemokine synthesis by human monocytes. The Journal of clinical investigation. 1996, 97: 1525-1534.

70. Neumann F J, Marx N, Gawaz M, Brand K, Ott I et al. Induction of cytokine expression in leukocytes by binding of thrombin-stimulated platelets. Circulation. 1997, 95: 2387-2394.

71. Abele S, Spriewald B M, Ramsperger-Gleixner M, Wollin M, Hiemann N E et al. Attenuation of transplant arteriosclerosis with clopidogrel is associated with a reduction of infiltrating dendritic cells and macrophages in murine aortic allografts. Transplantation. 2009, 87: 207-216.

72. Weyrich A S, Zimmerman G A. Platelets: signaling cells in the immune continuum. Trends in immunology. 2004, 25: 489-495.

73. Sharma B, Sharma M, Majumder M, Steier W, Sangal A, Kalawar M. Thrombocytopenia in septic shock patients--a prospective observational study of incidence, risk factors and correlation with clinical outcome. Anaesthesia and intensive care. 2007, 35: 874-880.

74. Winning J, Reichel J, Eisenhut Y, Hamacher J, Kohl M et al. Anti-platelet drugs and outcome in severe infection: clinical impact and underlying mechanisms. Platelets. 2009, 20: 50-57.

75. Seidel M, Winning J, Claus RA, Bauer M, Losche W. Beneficial effect of clopidogrel in a mouse model of polymicrobial sepsis. Journal of thrombosis and haemostasis : JTH. 2009, 7: 1030-1032.

76. Winning J, Neumann J, Kohl M et al. Antiplatelet drugs and outcome in mixed admissions to an intensive care unit. Critical care medicine. 2010, 38: 32-37.

77. Sitia G, Aiolfi R, Di Lucia P et al. Antiplatelet therapy prevents hepatocellular carcinoma and improves survival in a mouse model of chronic hepatitis B. Proc Natl Acad Sci U S A. 2012, 109: E2165-E2172.

78. Libby P, Ridker P M, Maseri A. Inflammation and atherosclerosis. Circulation. 2002, 105: 1135-1143.

79. Biasucci L M, Biasillo G, Stefanelli A. Inflammatory markers, cholesterol and statins: pathophysiological role and clinical importance. Clinical chemistry and laboratory medicine : CCLM / FESCC 2010, 48: 1685-1691.

80. Andreas Pfützner, Thomas Schöndorf, Markolf Hanefeld, Thomas Forst. High-sensitivity C-reactive protein predicts cardiovascular risk in diabetic and nondiabetic patients: effects of insulin-sensitizing treatment with pioglitazone. Journal of diabetes science and technology. 2010, 4: 706-716.

81. Micallef M A, Garg M L. Anti-inflammatory and cardioprotective effects of n-3 polyunsaturated fatty acids and plant sterols in hyperlipidemic individuals. Atherosclerosis. 2009, 204: 476-482.

82. Ridker P M, Silvertown J D. Inflammation, C-reactive protein, and atherothrombosis. Journal of periodontology. 2008, 79: 1544-551.

83. Dibra A, Mehilli J, Braun S et al. Inflammatory response after intervention assessed by serial C-reactive protein measurements correlates with restenosis in patients treated with coronary stenting. American heart journal 2005;150:344-50.

84. Cekici Y, Korkmaz H, Celik A, Bilen M N, Bulut M et al. The effect of the duration of clopidogrel use on hsCRP levels after stenting the target vessel in patients with acute coronary syndrome. Clinical and investigative medicine Medecine clinique et experimentale 2011, 34: E211.

85. Hermann A, Rauch B H, Braun M, Schror K, Weber A A. Platelet CD40 ligand (CD40L)--subcellular localization, regulation of expression, and inhibition by clopidogrel. Platelets. 2001, 12: 74-82.

86. Judge H M, Buckland R J, Holgate C E, Storey RF. Glycoprotein IIb/IIIa and P2Y12 receptor antagonists yield additive inhibition of platelet aggregation, granule secretion, soluble CD40L release and procoagulant responses. Platelets. 2005, 16: 398-407.

87. Steinhubl S R, Badimon J J, Bhatt D L, Herbert J M, Luscher TF. Clinical evidence for anti-inflammatory effects of antiplatelet therapy in patients with atherothrombotic disease. Vascular medicine. 2007, 12: 113-122.

88. Serebruany V L, Midei M G, Meilman H, Malinin A I, Lowry D R. Platelet inhibition with prasugrel (CS-747) compared with clopidogrel in patients undergoing coronary stenting: the subset from the JUMBO study. Postgraduate medical journal. 2006, 82: 404-410.