

Effect of antiplatelet therapy on inflammatory markers in atherothrombotic patients

Joseph B. Muhlestein

Cardiovascular Department, Intermountain Medical Center, University of Utah School of Medicine, Salt Lake City, Utah, USA

Summary

Inflammation is central to the pathogenesis and progression of atherosclerosis and thrombosis, the underlying cause of major cardiovascular disease. Platelets, in addition to their role in haemostasis, play a key role in both thrombus formation and inflammation following vascular injury, especially atherosclerotic lesions. An increasing body of evidence suggests that inhibition of platelet function can modulate inflammatory markers, particularly those associated with activated platelets, such as CD40 ligand, P-selectin, and C-reactive protein. The currently available antiplatelet agents aspirin, clopidogrel, prasugrel, abciximab, and eptifibatid have shown varying effects on inflammatory markers. These effects seem to be mostly indirect, i.e. mediated primarily through reduced platelet activation that results in reduced inflammatory marker expression. However, there is some evidence that suggests

direct effects (i.e. those independent of platelets) may also play a role in modulating inflammatory markers. Evidence linking inflammation and thrombosis supports the hypothesis that agents with both anti-inflammatory and antiplatelet effects may reduce vascular inflammation and limit acute and long-term thrombotic events. An assessment of the involvement of inflammatory mediators in atherosclerosis may provide further insight into important predictive markers of cardiovascular outcomes that may also serve as potential therapeutic targets. This review examines the evidence for and potential clinical relevance of the effects of antiplatelet therapy on inflammatory markers.

Keywords

Antiplatelet therapy, thrombosis, inflammation, platelets

Correspondence to:

Joseph B. Muhlestein, MD
University of Utah, University Hospital and Clinics
50 North Medical Drive
Salt Lake City, UT 84132, USA
Tel.: +1 801 507 4760, Fax: +1 801 507 4789
E-mail: brent.muhlestein@intermountainmail.org

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Introduction

Thrombosis is the body's natural clotting mechanism in response to blood vessel injury. Thus, it is also the response to disrupted atherosclerotic plaques caused by chronic inflammation of the intima of large arteries (1). Recruitment of T cells and macrophages to the site of plaque formation is central to the chronic inflammation underlying atherosclerosis and contributes to plaque instability (1, 2). Several factors contribute to intimal inflammation, including macrophage internalization of modified low-density lipoproteins (LDL) leading to activation of foam cells and recruitment of monocytes, macrophages, and additional LDL molecules; increased smooth-muscle lipoxygenase activity and formation of hydrogen peroxide and free radicals secondary to hypertension or smoking, all of which damage the endothelium and lead to white blood cell recruitment; acute or chronic infection with microorganisms; and platelet-mediated recruitment of monocytes (1–3). The formation of a platelet-rich thrombus at the site of disrupted atherosclerotic plaques, termed atherothrombosis, can lead to ischaemic events including acute coronary syn-

drome (ACS), ischaemic stroke, transient ischaemic attack (TIA), and critical limb ischaemia if the thrombus is large enough to block blood flow. Because inflammation is central to atherothrombosis, agents with both anti-inflammatory and antithrombotic properties may be critical to preventing the considerable morbidity and mortality associated with atherothrombotic vascular disease.

Platelets respond to both immunological and thrombotic stimuli (4) and thus play a key role in both inflammation and thrombus formation (► Fig. 1). The disruption of atherosclerotic plaques exposes their thrombogenic, lipid-rich core and promotes platelet adhesion to von Willebrand factor (vWF) and subendothelial collagen via glycoprotein receptors (5–8). Once exposed to local agonists, including adenosine diphosphate (ADP), serotonin, thromboxane A₂ (TxA₂), thrombin, and collagen, platelet activation and degranulation ensues. During this process, the expression of many surface receptors, including glycoprotein receptors and integrins, is upregulated, and conformational changes occur that facilitate the binding of soluble fibrinogen and vWF. Through the bridging effect, whereby a single fibrinogen

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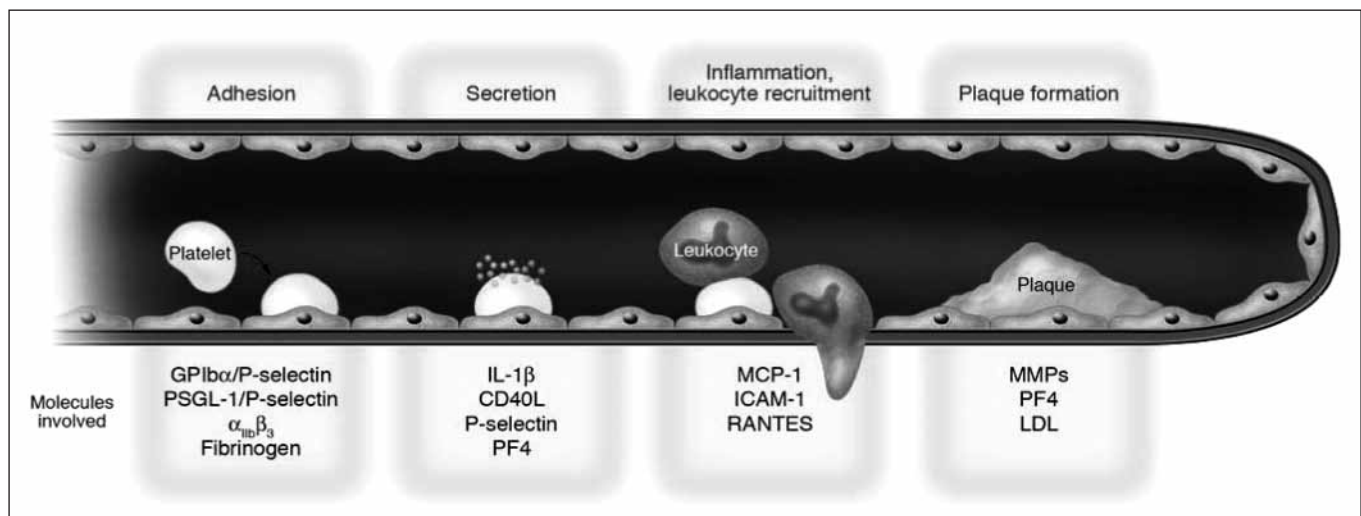


Figure 1: Hypothetical model of atherosclerosis triggered by platelets. Reproduced with permission from Gawaz et al. *J Clin Invest* 2005; 115: 3378–3384. Copyright 2005 by American Society for Clinical Investigation.

molecule is able to bind to two platelets, large platelet aggregates are formed.

As part of degranulation, the α -granules of activated platelets secrete inflammatory chemokines, cytokines, and growth factors (5). Although not an exhaustive list, secreted molecules include platelet-derived growth factor (PDGF); platelet factor 4 (PF4); CD40 ligand (CD40L); CD62P (P-selectin); the cytokine regulated upon activation, normal T-cell expressed, and secreted (RANTES); and transforming growth factor (TGF)- β (9) (► Table 1). As illustrated in Figure 1, these factors mediate the binding of platelets to leukocytes to form platelet-leukocyte aggregates (PLA), induce endothelial inflammation and the release of pro-inflammatory cytokines and chemokines, such as monocyte chemoattractant protein-1 (MCP-1) and interleukin (IL)-1 β , and induce platelet-endothelial and leukocyte-endothelial interactions, processes pivotal to thrombus formation and atherosclerosis (9–11). For example, CD40L and its receptor CD40 are co-expressed on activated platelets, vascular endothelial cells, smooth muscle cells, and monocytes in atherosclerotic lesions (12), allowing for thrombus propagation (Table 1). Similarly, the transmembrane protein P-selectin, when expressed on platelets, mediates the initial tethering of platelets to the vessel wall and the formation of PLAs that contribute to thrombosis and inflammation through binding to P-selectin glycoprotein ligand 1 (PSGL-1) on neutrophils and monocytes (13) (Table 1). Endothelial cells and platelets also bind to one another via this interaction, although in this case, endothelial cell-expressed P-selectin binds to platelet-expressed PSGL-1 (14).

Another result of platelet activation is induction of the coagulation cascade. One way in which platelets contribute to coagulation is by inducing procoagulant tissue factor expression on endothelial cells through CD40/CD40L and P-selectin/PSGL-1 interactions (15), thus activating the extrinsic coagulation pathway. Activated platelets also express phosphatidylserine, allowing them to bind the coagulation cofactor/enzyme complexes VIIa/IXa and Va/

Xa and initiate the intrinsic coagulation pathway (16). Coagulation factors are also able to associate with platelet aggregates through fibrinogen binding (17). As a result of initiating the coagulation cascade, thrombin is produced, which cleaves fibrinogen to fibrin and results in the formation of a platelet-containing clot. Additionally, thrombin amplifies platelet activation by increasing the expression of phosphatidylserine at the platelet surface, allowing the binding of additional coagulation co-factors and the production of more thrombin (18). Thus, activated platelets appear to be the common thread that links inflammation, thrombosis, and coagulation (19, 20).

The central role of inflammation in the pathogenesis of atherothrombosis highlights the importance of inflammatory markers as prognostic tools. Several inflammatory markers, including but not limited to CD40L (21–24), P-selectin (25), C-reactive protein (CRP) (26–29), tumour necrosis factor (TNF)- α (30, 31), MCP-1 (32, 33), IL-6 (26, 30) and intercellular cell adhesion molecule-1 (ICAM-1) (26, 34–37), are associated with atherosclerotic lesion progression and thrombosis, an increased risk of ischaemic events, and unfavourable short- and long-term outcomes across the spectrum of cardiovascular disease (Table 1). Elevated CD40L levels have been associated with an increased risk of cardiovascular events in patients with ACS (21) and apparently healthy women (22). Elevated levels of soluble and membrane-bound forms of CD40L in stable patients after percutaneous coronary intervention (PCI), and even higher levels in those with unstable angina (24) and ACS (38), suggest that the CD40L-CD40 interaction plays a pathogenic role in both the long-term atherosclerotic process and the induction and persistence of ACS. Elevated CRP levels have been found in patients with both stable and unstable angina (39) and have been shown to be an independent predictor of future ischaemic events (28). Levels of soluble ICAM-1 are higher in healthy individuals who subsequently go on to develop symptomatic arterial disease compared with those who do not (35, 37).

Table 1: Selected biomarkers associated with inflammation in atherothrombosis.

Name	General description	Potential role in atherothrombosis	Cellular source
CD40 ligand (CD40L) (23, 100, 101)	Member of the TNF family of molecules; expressed on activated T cells; involved in humoral immune response	Increases chemokine secretion and expression of adhesion molecules by EC; mediates attachment of neutrophils, monocytes, and lymphocytes to vessel wall; stimulates matrix degradation and contributes to plaque instability	Platelets, monocytes, T cells, NK cells, EC, SMC
C-reactive protein (CRP) (55, 102–104)	Plasma protein produced by liver and adipocytes in response to IL-1, IL-6, and TNF- α ; binds to foreign/damaged cells to aid phagocytosis by macrophages	Increases adhesion molecule expression and chemokine secretion by EC; inhibits endothelial NO and prostacyclin production, leading to EC apoptosis and inhibition of angiogenesis and progenitor cell survival; contributes to plaque formation by stimulating vascular smooth-muscle migration, proliferation and neointimal formation; contributes to plaque destabilization by stimulating matrix degradation; also stimulates proteins with known anti-inflammatory effects	Hepatocytes, adipocytes
Interleukin-1 β (IL-1 β) (10, 100, 101)	Produced by macrophages, monocytes, and dendritic cells; increases expression of endothelial adhesion factors; promotes proliferation of bone marrow cells; induces fever through its effect on the hypothalamus thermoregulatory center	Promotes EC activation and secretion of IL-6 and IL-8; increases expression of adhesion molecules to enable leukocyte transmigration into arterial intima; promotes expression of early inflammatory genes	Macrophages, lymphocytes, platelets, EC, SMC
Interleukin-6 (IL-6) (10, 105)	T _H cytokine that produces hematologic, immune, hepatic, endocrine, and metabolic effects	Promotes SMC proliferation; induces production of acute phase proteins, particularly CRP; induces differentiation of myeloid cells; increases platelet production and thrombogenicity; may be considered anti-inflammatory as it induces expression of proteins that downregulate inflammatory markers	EC, macrophages, SMC, T _H 2 cells
Interleukin-10 (IL-10) (106, 107)	T _H 2 cytokine involved in cross-regulation of T _H 1 response; enhances B-cell survival, proliferation, and antibody production	Downregulates release of pro-inflammatory cytokines and chemotactic factors; inhibits adhesion of monocytes to endothelium; promotes plaque stability through inhibition of matrix degradation	Monocytes, T _H cells
Intercellular cell adhesion molecule-1 (ICAM-1) (100, 101, 108)	Adhesion molecule found in low concentrations in the membranes of leukocytes and endothelial cells; induced by IL-1 and TNF- α	Promotes binding of activated platelets to endothelium; aids recruitment of leukocytes to inflamed tissue and subsequent transmigration into arterial intima	EC, leukocytes, platelets
Monocyte chemoattractant protein-1 (MCP-1) (100, 109)	Small cytokine belonging to the CC chemokine family; recruits monocytes, memory T cells, and dendritic cells to sites of tissue injury and infection	Chemoattractant—recruits monocytes and T cells to inflamed tissue	EC, monocytes
Platelet-derived growth factor (PDGF) (11)	Growth factor essential to cell growth, proliferation, and migration; also important in embryonic development and angiogenesis	Mediates the binding of leukocytes to platelets and endothelium	Platelets
Platelet factor-4 (PF4) (100, 101)	Small cytokine released from activated platelets during aggregation; promotes blood coagulation; inhibits endothelial cell proliferation and migration and may play a role in wound repair	Chemoattractant—recruits neutrophils, fibroblasts, and monocytes; induces neutrophil adhesion to endothelium and macrophage differentiation; promotes retention of lipoproteins and uptake of oxidized LDL by macrophages	Platelets
P-selectin (CD62P) (13, 100, 101)	Cell adhesion molecule found in endothelial cells and platelets	Strengthens initial rolling contact between platelets and vessel wall; recruits neutrophils, monocytes, and T cells to inflamed tissue; promotes deposition of platelet-derived RANTES (increases recruitment of monocytes to the endothelium)	Platelets, EC
RANTES (100, 101)	Chemokine active in recruiting leukocytes to inflammatory sites	Chemoattractant—recruits monocytes to inflamed endothelium (in conjunction with P-selectin); enhances T-cell recruitment	Platelets

Table 1: Continued

Name	General description	Potential role in atherothrombosis	Cellular source
Transforming growth factor- β (TGF- β) (10)	Anti-inflammatory cytokine involved in cellular proliferation and differentiation; important for tissue regeneration, wound healing, angiogenesis	Balances action of pro-inflammatory cytokines; protects against loss of collagen and matrix degradation to prevent plaque rupture; induces regulatory immunity	Platelets, macrophages, T _H cells, B cells, SMC
Tumour necrosis factor- α (TNF- α) (10)	Cytokine involved in diverse intercellular signalling pathways, including systemic inflammation, apoptotic cell death, and tumourigenesis	Mediates expression of cellular adhesion molecules and promotes leukocyte transmigration through restructuring of intercellular junctions; activates neutrophils; induces secretion of other inflammatory cytokines	Macrophages, T cells, B cells, NK cells, SMC

EC, endothelial cells; IL, interleukin; LDL, low-density lipoprotein; NK, natural killer; NO, nitric oxide; T_H, T-helper; RANTES, regulated upon activation, normal T-cell expressed, and secreted; SMC, smooth muscle cells; TNF, tumour necrosis factor.

Monocyte chemoattractant protein-1, which is highly expressed in human atherosclerotic lesions (32, 40), is found to be elevated in peripheral arterial disease (PAD) patients and associated with an increased risk of coronary heart disease (33). In contrast to most other cytokines, IL-10 has anti-inflammatory properties (Table 1), and elevated plasma levels have been associated with a reduced incidence of cardiovascular events (41).

A growing body of evidence suggests that long-term antiplatelet therapy may exert benefit not only by reducing platelet activation, but also by reducing inflammation (42). The purpose of this review is to examine the evidence for the effects of antiplatelet therapy on inflammatory markers and discuss the potential clinical relevance of those effects.

Antiplatelet therapy and inflammatory markers

Due to the close association between inflammation, atherosclerosis, and thrombosis, it is believed that effective and sustained platelet inhibition may not only provide an antithrombotic effect, but also favourably impact atherosclerotic plaque stability (43). Initial evidence for the potential anti-inflammatory benefits of antiplatelet agents comes from both *in vitro* and *in vivo* preclinical studies. *In vitro* experiments using human platelets and endothelial cells demonstrated that the antiplatelet agents clopidogrel and prasugrel are able to inhibit expression of CD40L and P-selectin and reduce PLA formation (44–48). In animal models, antiplatelet therapy with clopidogrel or an inhibitor of the TxA₂ receptor was associated with reduced expression of inflammatory markers such as P-selectin, CD40L, tissue factor, and CRP, and improved endothelial cell function (43, 49–52). Therefore, mounting evidence points to a promising therapeutic, anti-inflammatory role for the guideline-approved antiplatelet agents aspirin, clopidogrel, prasugrel, and glycoprotein IIb/IIIa inhibitors (► Table 2).

Aspirin

Aspirin was the first antiplatelet agent used for both the primary and secondary prevention of cardiovascular events. By irreversibly binding to cyclooxygenase (COX)-1, aspirin inhibits TxA₂ production, thus blocking TxA₂-induced platelet aggregation and vasoconstriction (53). In *in vitro* studies, aspirin inhibits nuclear factor (NF)- κ B-dependent induction of adhesion molecules in endothelial cells and subsequent monocyte adhesion, suggesting this may be another mechanism through which aspirin acts as an anti-inflammatory agent (54).

Evidence from clinical studies shows that aspirin has effects on critical inflammatory markers (Table 2). In the Physician's Health Study, a plasma CRP level = 1.15 mg/l was an independent predictor of myocardial infarction and stroke in apparently healthy men at baseline (55). Although the risk of myocardial infarction increased at all quartiles of CRP levels regardless of whether the patients received aspirin or placebo, rates were lower in aspirin recipients. Furthermore, the benefits of aspirin increased linearly with CRP levels such that patients with the highest baseline CRP levels derived the greatest benefit from aspirin (55). The ability of aspirin to reduce CRP levels has been confirmed in other patient populations, including those with stable coronary artery disease (CAD) (31, 56) and those with ACS (57, 58). Aspirin has also been shown to reduce levels of IL-6, MCP-1, TGF- β , and TNF- α (31, 57, 58). Surprisingly, aspirin does not seem to affect platelet-leukocyte interactions (59). As some of these effects are seen with doses of aspirin below that which is thought to be directly anti-inflammatory, the benefit is likely due to aspirin's indirect effects on platelets.

Clopidogrel

The thienopyridine clopidogrel selectively and irreversibly inhibits platelet ADP P2Y₁₂ receptors, thereby inhibiting agonist-induced platelet aggregation. In clinical trials and compared with aspirin alone, clopidogrel monotherapy (60) and combination therapy

Table 2: Effects of antiplatelet therapy on selected inflammatory biomarkers.

Study	Population and duration of therapy	Treatment	Marker(s) assessed	Results
Aspirin or Clopidogrel monotherapy				
Prospectively designed substudy of the randomised, double-blind Physician's Health Study (55)	Healthy men who developed MI, stroke, or venous thrombosis during the study (n = 543) Healthy men who did not develop vascular disease during the study (n = 543) Duration = Up to 8 years	Aspirin vs. Placebo	Plasma CRP	Aspirin significantly reduced the risk of MI, with the magnitude of benefit increasing along with the plasma CRP level (P = 0.02)
Prospective, randomised crossover study (56)	Patients with chronic stable angina (n = 60) Duration = 6 weeks	Aspirin vs. Placebo	Plasma CRP, M-CSF, IL-6, and IL-1b	Plasma CRP, IL-6, and M-CFF levels significantly reduced in aspirin recipients at 6 weeks (P < 0.05 for each)
Prospectively designed substudy of the randomised, open-design WARIS II trial (58)	Patients with AMI (n = 310) Duration = 4 years	Aspirin vs. Aspirin + Warfarin vs. Warfarin	Serum hsCRP, TNF- α , IL-6, and IL-10	Serum hsCRP levels significantly lower in ASA-treated patients at 3 months (P = 0.032) and 4 years (P = 0.023) Serum IL-6 levels significantly lower in ASA-treated patients at 4 years (P = 0.034) but not at 3 months (P = 0.130) No significant between-treatment differences in levels of serum TNF- α or IL-10
Prospectively designed substudy of the randomised, open-design ASCET trial (31)	Patients with stable CAD (n = 206) Duration = 1 year	Aspirin vs. Clopidogrel	Serum hsCRP, TNF- α , IL-6, and IL-10 Plasma MCP-1, P-selectin, and CD40L	No significant between-treatment differences noted at 1 month or 1 year Serum TNF- α and plasma MCP-1 levels significantly lower at 1 year in aspirin recipients (P < 0.001 for each) Serum TNF- α levels significantly lower at 1 year in clopidogrel recipients (P < 0.001)
Prospective, non-randomised study (67)	Healthy volunteers (n = 10) Duration = 6 days	Clopidogrel	Whole blood PLA Platelet P-selectin	TRAP- and ADP-induced expression of P-selectin and formation of PLA significantly reduced by clopidogrel (P < 0.03 for all)
Aspirin + Clopidogrel				
Prospective, non-randomised study (75)	PCI patients (n = 74) ● 31 pretreated with clopidogrel ● 43 not pretreated with clopidogrel Duration = 24 hours	Clopidogrel ^a vs. No Clopidogrel ^a	Platelet CD40L and P-selectin Serum sCD40L and IL-6	Platelet CD40L levels significantly lower in clopidogrel recipients at baseline and post-procedure (P = 0.002 and P = 0.0007, respectively) Platelet P-selectin levels significantly lower in clopidogrel recipients at baseline, post-procedure, and 18–24 hours (P < 0.001, P = 0.001, and P = 0.03, respectively) Serum sCD40L levels significantly lower in clopidogrel recipients post-procedure (P = 0.04) No significant post-procedure differences in serum IL-6 levels
Prospective, non-randomised study (110)	Patients with NSTEMI (n = 23) Duration = 24 hours	Clopidogrel loading dose ^b (300 mg)	Whole blood PLA and P-selectin Plasma sCD40L and P-selectin	ADP- and TRAP-induced P-selectin levels (whole blood) significantly reduced by clopidogrel (P < 0.01) Plasma sCD40L and P-selectin levels significantly reduced by clopidogrel (P < 0.001 for each) Whole blood PLA formation significantly reduced by clopidogrel (P < 0.01)

Table 2: Continued

Study	Population and duration of therapy	Treatment	Marker(s) assessed	Results
Aspirin + Clopidogrel				
Prospective, non-randomised study of patients enrolled in a PCI registry (74)	Consecutive PCI patients (n = 833) Median duration = 5 days	Clopidogrel loading dose ^c (300–600 mg)	Serum hsCRP	Periprocedural rise in hsCRP significantly reduced by clopidogrel (P = 0.03)
ALBION (randomised, double-blind, parallel group study) (77)	NSTEMI patients (n = 103) Duration = 24 hours	Clopidogrel loading dose ^d (300 mg vs. 600 mg vs. 900 mg)	Serum hsCRP and sCD40L Plasma PAI-1 and vWF	No significant between-treatment differences
Prospective, randomised, double-blind study (57)	Patients with NSTEMI (n = 115) Duration = 30 days	Aspirin vs. Aspirin + Clopidogrel	Serum hsCRP and TNF- α	Levels of serum hsCRP and TNF- α significantly reduced after 7 and 30 days of aspirin and aspirin + clopidogrel treatment (P < 0.01 for all) 7- and 30-day serum hsCRP and 30-day serum TNF- α levels significantly lower in aspirin + clopidogrel recipients (P < 0.05 for all)
Prospective, randomised, single-blind study (72)	NSTEMI patients (n = 86) Duration = 36 weeks	Clopidogrel ^d vs. No Clopidogrel ^d	Serum P-selectin, hsCRP, and sCD40L	Mean P-selectin levels significantly lower in aspirin + clopidogrel recipients with elevated hsCRP and sCD40L levels (P = 0.018)
Prospective, non-randomised study (71)	Consecutive patients with stable CHD (n = 103) Duration = 5 weeks	Aspirin + Placebo vs. Aspirin + Clopidogrel	Plasma sCD40L, RANTES, and hsCRP	Plasma levels of sCD40L, RANTES, and hsCRP significantly reduced in aspirin + clopidogrel recipients (P < 0.05, P < 0.01, and P < 0.01, respectively)
Prospective, randomised, double-blind trial (70)	Patients with stable CHD (n = 73) Duration = 8 weeks	Aspirin + Placebo vs. Aspirin + Clopidogrel	Plasma sCD40L and hsCRP	Plasma CD40L levels significantly reduced in aspirin + clopidogrel recipients (P = 0.03) Plasma hsCRP levels not significantly reduced in aspirin + clopidogrel recipients
Prospective, non-randomised, cross-sectional study (68)	PAD patients (n = 44) Healthy volunteers (n = 9)	Aspirin vs. Clopidogrel vs. Clopidogrel + Aspirin	Platelet-rich plasma PLA, P-selectin, MAC-1, and ICAM-1	TRAP- or ADP-induced platelet P-selectin and MAC-1 expression and PLA formation significantly reduced by clopidogrel and clopidogrel + aspirin (P < 0.05 for all) Soluble plasma ICAM-1 levels significantly reduced by clopidogrel, aspirin, and clopidogrel + aspirin (P < 0.05 for all)
Prospective, non-randomised study (69)	PAD patients (n = 26) Duration = 2 weeks	Aspirin vs. Clopidogrel vs. Cilostazol vs. Clopidogrel + Aspirin vs. Clopidogrel + Cilostazol vs. Aspirin + Clopidogrel + Cilostazol	Whole blood TF-PCA Plasma FVIIa, TAT, F1.2, P-selectin, CRP, and sCD40L	Whole blood TF-PCA levels significantly reduced in clopidogrel (P = 0.02), clopidogrel + aspirin (P = 0.02), clopidogrel + cilostazol (P = 0.004), and clopidogrel + aspirin + cilostazol (P < 0.001) recipients Plasma P-selectin levels significantly reduced by aspirin, cilostazol, and clopidogrel and all combinations thereof (P < 0.001 for all) No other significant between-treatment differences

Table 2: Continued

Study	Population and duration of therapy	Treatment	Marker(s) assessed	Results
Aspirin + Prasugrel				
Prospectively designed substudy of the randomised, double-blind JUMBO trial (81)	Coronary stent recipients (n = 131) Duration = 30 days	Prasugrel + Aspirin ^e vs. Clopidogrel + Aspirin ^e	Whole blood P-selectin and CD40L	P-selectin and CD40L levels significantly lower in prasugrel recipients at 24 hours and 30 days (P < 0.05 for all)
Prospective, randomised, double-blind study (82)	Patients with stable CAD (n = 110) Duration = 29 days	Prasugrel + Aspirin vs. Clopidogrel + Aspirin	Whole blood P-selectin and PLA formation	P-selectin levels significantly lower in prasugrel recipients at 2 and 24 hours and 14 and 29 days (P < 0.01 for all) PLA formation significantly lower in prasugrel recipients at 2 and 24 hours and 14 and 29 days (P < 0.001 for all)
Glycoprotein IIb/IIIa Inhibitors				
Prospectively designed substudy of the randomised, double-blind EPIC trial (88)	Patients undergoing angioplasty (n = 160) Duration = 4 weeks	Abciximab ^b vs. Placebo ^b	Plasma CRP, IL-6, and TNF- α	Plasma CRP and IL-6 levels rose significantly less at 24–48 hours in abciximab recipients (P = 0.025 and P < 0.001, respectively) No significant between-treatment differences at 4 weeks
Prospective, non-randomised study (89)	Consecutive stent recipients (n = 50) Duration = 48 hours	Eptifibatide ^f vs. No Eptifibatide ^f	Serum CRP and IL-6	Serum CRP levels rose significantly less at 24 hours in eptifibatide recipients (P < 0.05) No significant between-treatment differences in serum CRP at 48 hours or serum IL-6 levels at 24 or 48 hours

^aAll patients received aspirin plus heparin or bivalirudin. ^bAll patients received aspirin plus heparin. ^cAll patients received aspirin. ^dAll patients received aspirin plus low-molecular-weight heparin. ^eThere were 7 aspirin + prasugrel recipients derived from the JUMBO trial and 124 aspirin + clopidogrel recipients derived from a comparable historic cohort. ^fAll patients received aspirin plus heparin plus clopidogrel. ADP, adenosine diphosphate; ALBION, Assessment of the best Loading dose of clopidogrel to Blunt platelet activation, Inflammation, and Ongoing Necrosis; AMI, acute myocardial infarction; ASA, aspirin; ASCET, Aspirin non-responsiveness and Clopidogrel Endpoint Trial; CAD, coronary artery disease; CD40L, CD40 ligand; CHD, coronary heart disease; CRP, C-reactive protein; CV, cardiovascular; EPIC, Evaluation of c7E3 for the Prevention of Ischemic Complications; ESPRIT, Enhanced Suppression of the

Platelet IIb/IIIa Receptor with Integrilin Therapy; FVIIa, factor VIIa; hsCRP, high-sensitivity CRP; ICAM-1, intercellular cell adhesion molecule-1; IL, interleukin; JUMBO, Joint Utilization of Medications to Block platelets Optimally; MAC-1, monocyte CD11b; M-CSF, macrophage colony-stimulating factor; MI, myocardial infarction; MCP-1, monocyte chemoattractant protein-1; NSTEMI, non-ST-elevation myocardial infarction; PAD, peripheral arterial disease; PAI-1, plasminogen activator inhibitor-1; PCI, percutaneous coronary intervention; PLA, platelet-leukocyte aggregate; P-SEL, P-selectin; RANTES, regulated upon activation, normal T-cell expressed, and secreted; TF-PCA, tissue factor procoagulant activity; TAT, thrombin-antithrombin complexes; TGF- β , transforming growth factor- β ; TNF- α , tumour necrosis factor- α ; TRAP, thrombin receptor-activating peptide; vWF, von Willebrand factor; WARIS II, Warfarin Aspirin Reinfarction Study II.

with aspirin (61–66) have both been shown to significantly decrease the risk of adverse cardiovascular events in patients with symptomatic ischaemic vascular disease. An increasing body of evidence shows that clopidogrel reduces the expression of inflammatory markers in patients with ischaemic vascular disease (Table 2). As clopidogrel has no known direct anti-inflammatory action, this suggests that its clinical benefit may be due to indirect anti-inflammatory effects in addition to its well-characterised antiplatelet activity.

In a substudy of the Aspirin non-responsiveness and Clopidogrel Endpoint Trial (ASCET), patients with stable CAD had comparable reductions in the inflammatory markers CRP, TNF- α , IL-6, IL-10, MCP-1, CD40L, P-selectin, and TGF- β at one year, regardless of whether they received aspirin or clopidogrel (31). It

should be noted that all participants in this study were taking aspirin at the time of randomisation; whether this affected the reduction in levels of inflammatory markers, particularly TNF- α , is not clear. In healthy volunteers (67) and patients with PAD (68), clopidogrel reduced the formation of PLA through attenuation of P-selectin expression. In the study conducted among PAD patients, clopidogrel also reduced agonist-induced monocyte CD11b expression on monocytes, thus indirectly reducing inflammation by reducing leukocyte activation by adherent platelets (68). In another study conducted in PAD patients, clopidogrel was able to reduce tissue factor procoagulant activity, suggesting another mechanism through which clopidogrel might exert its clinical benefit (69).

Aspirin plus clopidogrel

Results of several studies conducted in healthy volunteers and patients with stable ischaemic vascular disease show that combinations of antiplatelet agents reduce inflammatory markers to a greater extent than single agents (68–72) (Table 2). In two studies of patients with stable CAD who were randomised to receive clopidogrel or placebo in addition to aspirin, platelet-derived sCD40L was reduced only in those who received clopidogrel (70, 71). In a study performed by Heitzer et al., the addition of clopidogrel to aspirin reduced high-sensitivity CRP (hsCRP) and RANTES levels, improved endothelial nitric oxide bioavailability, and reduced markers of oxidant stress (60). While Azar et al. observed a 17% reduction in sCD40L levels in stable CAD patients treated with clopidogrel, they did not find a similar reduction in hsCRP levels (70). The investigators commented that the reduction in sCD40L may have been too modest to have translated into a reduction in hsCRP, a measure of subclinical inflammation. As shown in a study of patients with PAD, combining clopidogrel with aspirin and cilostazol, an antiplatelet agent used to treat claudication, provided greater benefit than clopidogrel alone (69).

The combination of aspirin and clopidogrel is also effective in patients with ACS or those undergoing PCI (57, 72–76) (Table 2). In a study of non-ST-segment elevation myocardial infarction (NSTEMI) patients, reductions of hsCRP and TNF- α levels at 30 days were significantly greater in patients treated with aspirin and clopidogrel compared to aspirin alone (57). In another, smaller study of NSTEMI patients with highly activated platelets, hsCRP and sCD40L levels were reduced only in patients who received clopidogrel in addition to aspirin (72). As assessed by P-selectin levels 8 hours (h) post-treatment, addition of clopidogrel to aspirin significantly reduced early platelet activation compared with aspirin alone and was associated with protection against recurrent ischaemic events at 52 weeks.

Among patients undergoing PCI, one large study demonstrated that despite similar baseline CRP levels, the periprocedural rise in CRP was 65% lower ($p = 0.03$) in those pre-treated with clopidogrel versus those who were not (74). Multivariate linear regression analysis showed that clopidogrel pre-treatment was an independent predictor of post-PCI attenuation of CRP. In another study of patients undergoing PCI, clopidogrel pre-treatment significantly and independently reduced platelet expression of CD40L and P-selectin both before and after the procedure compared to those who received no pre-treatment (75). Of note, these reductions were associated with reduced IL-6 levels, suggesting clopidogrel may mediate downstream anti-inflammatory effects. The effects of long-term clopidogrel therapy on inflammatory markers are less clear. Results of the Evaluation of Long-term clopidogrel AntiPlatelet and Systemic anti-inflammatory Effects (ELAPSE) study, a prospective study of 26 patients who received clopidogrel and aspirin prior to coronary stenting and for 12 months thereafter, showed that P-selectin levels were significantly increased over baseline at 12 months while soluble CD40L levels were significantly decreased at both six and 12 months (76). In their discussion of the ELAPSE results, the investigators acknowledge that their findings are some-

what confusing and recommend that additional studies be conducted in larger patient populations.

There is compelling evidence that clopidogrel loading doses greater than the standard 300 mg are associated with a significantly faster onset of platelet inhibition (77, 78) and a reduction in ischaemic complications (79). In a study comparing clopidogrel loading doses of 300 and 450 mg in patients with CAD, the higher dose was associated with more rapid platelet inhibition and reduced expression of platelet-bound P-selectin, but not with a change in soluble P-selectin levels (78). The Assessment of the best Loading dose of clopidogrel to Blunt platelet activation, Inflammation, and Ongoing Necrosis (ALBION) trial, conducted in NSTEMI patients receiving standard treatment including aspirin, demonstrated greater attenuation of hsCRP levels within 24 h when a clopidogrel loading dose of either 600 or 900 mg was administered instead of the standard 300 mg dose (77). However, this difference was not significant and not apparent at 6 h, and no such dose response was seen for reductions in levels of the other inflammatory markers assessed. Furthermore, additional evidence indicates there is no further improvement in platelet inhibition with clopidogrel loading doses greater than 600 mg (80), suggesting that future studies conducted to confirm the antiplatelet and anti-inflammatory benefits of clopidogrel loading should focus on doses no greater than 600 mg.

Aspirin plus prasugrel

Similar to clopidogrel, prasugrel is a thienopyridine that inhibits agonist-induced platelet aggregation by irreversibly binding to platelet P2Y₁₂ receptors. Outside of *in vitro* studies conducted using the blood of healthy volunteers (44, 45), there are limited data on the effects of prasugrel on inflammatory markers (Table 2). In an analysis of seven prasugrel plus aspirin recipients who underwent coronary stenting secondary to ACS as part of the Joint Utilization of Medications to Block Platelets Optimally (JUMBO) trial, 24-h and 30-day levels of P-selectin and CD40L were significantly lower than baseline levels (81). Compared with a historical cohort of 124 comparable clopidogrel recipients, prasugrel recipients had significantly lower levels of P-selectin at both 24 h and 30 days and CD40L at 30 days. Similarly, in a randomised study of 110 aspirin recipients who had stable CAD, those who received prasugrel had significantly decreased PLA formation and significantly lower levels of P-selectin at 2 and 24 h and 14 and 29 days after initiation compared with those who received clopidogrel (82). The greater effect of prasugrel on inflammatory markers echoes the significantly greater degree of platelet inhibition induced by prasugrel compared with clopidogrel (83) and may contribute to its significant benefit in reducing the risk of thrombotic events in patients with ACS who undergo PCI (84). Interestingly, the active metabolite of prasugrel has been shown to inhibit procoagulant activity in *in vitro* studies conducted in blood from healthy volunteers (44, 85); if confirmed *in vivo*, this anticoagulant activity could be a contributing factor to the significantly increased risk of bleed-

ing observed in some patient subgroups receiving prasugrel versus clopidogrel.

Glycoprotein IIb/IIIa inhibitors

The glycoprotein IIb/IIIa receptor antagonists abciximab, eptifibatid, and tirofiban inhibit the glycoprotein IIb/IIIa integrin receptor on platelets, thereby inhibiting platelet aggregation and thrombus formation at the site of plaque disruption and markedly decreasing the risk of ischaemic complications post-PCI (53, 86, 87). Several clinical studies suggest that blockade of the glycoprotein IIb/IIIa receptor also limits the inflammatory response secondary to coronary intervention (Table 2). A retrospective analysis of the Evaluation of c7E3 for the Prevention of Ischemic Complications (EPIC) trial demonstrated that abciximab significantly suppressed the periprocedural rise in CRP, IL-6, and TNF- α observed in patients undergoing percutaneous coronary revascularisation (88). By four weeks, levels of inflammatory markers returned to baseline and there was no significant difference between patients treated with abciximab and those who were not. Overall, the study investigators concluded that the ability of abciximab to reduce inflammatory marker expression may contribute to its clinical benefit (88). Similar results were observed in a prospective study of 50 consecutive stent recipients, in which a 24-h infusion of eptifibatid suppressed the post-coronary angiography rise in CRP levels compared with standard antithrombotic therapy (89). However, as CRP levels rose within 48 h of eptifibatid cessation, the investigators proposed that a longer duration of infusion may be required to induce a meaningful anti-inflammatory effect in the absence of dual antiplatelet therapy with aspirin and clopidogrel (89). In contrast to the findings of the EPIC study, a substudy of the Swedish Global Use of Strategies to Open Occluded arteries (GUSTO) IV-ACS trial showed that the 24- to 48-h infusion of abciximab had no effect on the inflammatory markers IL-6, CRP, and fibrinogen (90). Whether this resulted from too low of an infusion dose or some other reason is not known. It should also be noted that abciximab infusions of 24 or 48 h are not recommended as they were associated with an increased risk of mortality in this trial (91).

Direct versus indirect effects of antiplatelet therapy

Two hypotheses, the direct and indirect, have been put forth to explain the anti-inflammatory effects mediated by antiplatelet agents. The indirect hypothesis suggests that the effects on inflammatory markers are secondary to decreased platelet activation. Studies in which inflammatory markers are reduced secondary to, or along with, platelet activation support the indirect hypothesis. For example, in the Clopidogrel Loading with Eptifibatid to Arrest the Reactivity of Platelets (CLEAR PLATELETS) 1b study, patients whose platelets were inhibited the most also showed the greatest in-

hibition of CRP (92). This hypothesis is also supported by those studies that illustrate a more pronounced effect of antiplatelet therapies on inflammatory markers in unstable or hyperreactive patients compared to patients with stable disease or healthy individuals. In a study conducted by Vavuranakis et al., the addition of clopidogrel to aspirin therapy inhibited early activation of platelets only in the subgroup of patients with ACS and highly activated platelets (defined as those with high hsCRP and sCD40L levels) (72). In patients with diabetes mellitus, a population known to have increased levels of platelet reactivity, clopidogrel withdrawal following long-term dual antiplatelet therapy was associated with an increase in both platelet and inflammatory biomarkers (93).

The direct hypothesis suggests that antiplatelet agents exert their effects directly on the inflammatory mediators themselves. Support for the direct hypothesis comes from studies demonstrating lowering of inflammatory markers among stable patients or healthy individuals taking antiplatelet therapy (56, 69, 94); the inhibition of NF- κ B, a transcription factor that regulates expression of genes involved in the immune response, by aspirin in human endothelial cell cultures (54), by clopidogrel in pigs that underwent PCI (95), and by glycoprotein IIb/IIIa inhibitors in stimulated neutrophils plated on fibronectin (96); and the clopidogrel-mediated transcriptional inhibition of several gene products involved in inflammation and cellular stress responses in a mouse model of endotoxaemia (97). Observations from a rabbit model of coronary infarction also support the direct hypothesis (52). In this study, levels of the inflammatory markers CD40L, endothelial nitric oxide synthase, and tissue factor were reduced almost completely to control levels within 24 h of clopidogrel administration, while platelet inhibition was only partially reduced in this timeframe.

The question of whether the anti-inflammatory effect of antiplatelet agents comes mainly from an indirect or direct effect cannot be known for certain from existing data. However, it is the opinion of this author that most of the anti-inflammatory effect of antiplatelet agents comes indirectly through the prevention of platelet activation. Some evidence supporting this hypothesis comes from the results of the Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance (CHARISMA) trial (98). In this study evaluating the effect of long-term use of clopidogrel in stable patients with cardiovascular disease, or at risk for such, already taking aspirin, clopidogrel did not provide significant clinical benefit for the overall population; pre-specified (98) and *post hoc* (99) analyses revealed that clopidogrel was clinically beneficial only in patients with documented cardiovascular disease, particularly those with a history of myocardial infarction, stroke, or symptomatic PAD. This may have been because these patients are more likely to have ongoing platelet activation that leads to an enhanced inflammatory state, which can be indirectly prevented by the antiplatelet effect of clopidogrel. On the other hand, if the majority of the anti-inflammatory effect from clopidogrel comes from a direct action on the inflammatory pathway, then one would expect a clinical reduction in future ischaemic events even among patients who had no evidence of ongoing or prior platelet activation, which was not the case in the CHARISMA trial.

	Aspirin	Clopidogrel	Aspirin plus Clopidogrel	Aspirin plus Prasugrel	GP IIb/IIIa inhibitors
CD40L		X	X	X	X
CRP	X		X		X
IL-6	X				X
MAC-1			X		
MCP-1	X				
M-CSF	X				
PLA formation		X	X	X	
P-selectin (CD62)		X	X	X	
TF-PCA			X		
TNF- α	X		X		

"X" indicates antiplatelet agent modifies formation/expression of marker. CD40L, CD40 ligand; CRP, C-reactive protein; GP, glycoprotein; IL-6, interleukin-6; MAC-1, monocyte CD11b; MCP-1, monocyte chemoattractant protein-1; M-CSF, macrophage colony-stimulating factor; PLA, platelet-leukocyte aggregate; TF-PCA, tissue factor procoagulant activity; TNF- α , tissue necrosis factor- α .

Table 3: Summary of antiplatelet effects on selected inflammatory markers in humans.

Conclusions

Vascular inflammation is an underlying mechanism central to atherosclerotic disease and the response to vascular injury. Data from animal and human model systems suggest that modification of platelet function may also modulate expression of known inflammatory mediators, including CD40L, CRP, and TNF- α , and the formation of PLA. Although the clinical studies, in which the anti-inflammatory effect of antiplatelet therapy was assessed, vary with regard to study design (i.e. randomised vs. non-randomised), the number of patients enrolled, whether they had stable or acute disease, and how the markers were measured (different assays and different biological materials [i.e. whole blood, platelet-rich plasma, serum]) (Table 2), the accumulated data support the hypothesis that antiplatelet therapies may reduce vascular inflammation in patients with ischaemic vascular disease. Therefore, antiplatelet therapy may help break the cycle of cardiovascular disease not only by inhibiting platelet activation and aggregation, but also by limiting local and systemic inflammatory amplification loops.

Results of clinical studies have shown that different antiplatelet agents may affect different inflammatory markers (►Table 3). Overall, the anti-inflammatory properties of antiplatelet agents are likely to be a result of decreased platelet activation and therefore, indirect. While an indirect hypothesis is supported by a number of studies, some data suggest that direct mechanisms may also play a role in decreasing inflammation. Future studies conducted with both established and novel antiplatelet agents may expand our understanding of the relationships between antiplatelet therapy and vascular inflammation, and thus their ability to decrease the risk of adverse ischaemic events.

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