Platelets, inflammation and anti-inflammatory effects of antiplatelet drugs in ACS and CAD

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Summary
Platelets play a pivotal role in chronic inflammation leading to progression of atherosclerosis and acute coronary events. Recent discoveries on novel mechanisms and platelet-dependent inflammatory targets underpin the role of platelets to maintain a chronic inflammatory condition in cardiovascular disease. There is strong and clinically relevant crosslink between chronic inflammation and platelet activation. Antiplatelet therapy is a cornerstone in the prevention and treatment of acute cardiovascular events. The benefit of antiplatelet agents has mainly been attributed to their direct anti-aggregatory impact. Some anti-inflammatory off-target effects have also been described. However, it is unclear whether these effects are secondary due to inhibition of platelet activation or are caused by direct distinct mechanisms interfering with inflammatory pathways. This article will highlight novel platelet associated targets that contribute to inflammation in cardiovascular disease and elucidate mechanisms by which currently available antiplatelet agents evolve anti-inflammatory capacities, in particular by carving out the differential mechanisms directly or indirectly affecting platelet mediated inflammation. It will further illustrate the prognostic impact of antiplatelet therapies by reducing inflammatory marker release in recent cardiovascular trials.

Keywords
Antiplatelet agents, atherosclerosis, cytokines, platelet physiology, inflammation

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Mechanisms of platelet mediated inflammation
Platelets are critically involved in haemostasis, act as repair and inflammatory cells and link the haemostatic and inflammatory system (1, 2). Platelets recruit circulating endothelial progenitor cells to sites of vascular injury and may induce a prothrombotic state by platelet-endothelium- and platelet-leucocyte-endothelium interactions (3). During adhesion, platelets are activated and release potent inflammatory and mitogenic substances into the local microenvironment, thereby altering function of endothelial cells (4). These alterations support chemotaxis, adhesion and transmigration of monocytes to the site of inflammation (5). Platelets secrete or expose adhesion proteins, growth factors, chemokines, cytokine-like factors and coagulation factors (6, 7). Once recruited to the vascular wall, platelets may promote inflammation via chemotraction of leukocytes, stimulate vascular smooth muscle cell (VSMC) proliferation and contribute to matrix degradation (6, 8). Certain chemokines enhance platelet aggregation and adhesion and trigger recruitment of monocytes (Table 1) (9). A candidate involved in monocyte recruitment is the CC chemokine regulated upon activation, normal T cell expressed and secreted (RANTES). Platelet RANTES release triggers monocyte arrest on inflamed or atherosclerotic endothelium (10). The most abundantly secreted protein by activated platelets is the chemokine platelet factor 4 (PF4). PF4 induces the differentiation of monocytes into macrophages that upregulate both, toll-like receptors (TLRs) involved in macrophage activation and scavenger receptors (SRs) (11). PF4 directly contributes to proatherogenic actions by promoting retention of lipoproteins (12). PF4 also interacts directly with oxidised low-density lipoprotein (oxLDL) leading to an induction of endothelial cell activation and dysfunction, macrophage foam cell formation, and VSMC migration and proliferation (13). Vascular wall cells express several SRs on their surface that mediate cellular effects of oxLDL. The lectin-like oxidised low-density lipoprotein receptor-1 (LOX-1) is the main oxLDL receptor of endothelial cells. It is also expressed in macrophages and VSMCs. Macrophages internalise apoptotic cell fragments, bacterial endotoxins, and oxLDL, leading to lipid accumulation and foam cell formation. Interestingly, PF4 in macrophages correlates with the presence and the extension of atherosclerotic lesions (13, 14). CD40 ligand (CD40L, CD154) and its receptor CD40 are co-stimulatory molecules of the tumour necrosis factor (TNF) and TNF receptor family. They play important roles in modulating
immune responses and inflammation. CD40L is expressed on various cell types present in or around atherosclerotic plaques, such as T cells, macrophages, VSMCs, endothelial cells, and platelets. Activated platelets are the most important source of circulating, soluble CD40L. Platelet CD40L also induces secretion of chemokines and expression of adhesion molecules in endothelial cells leading to leucocyte recruitment towards the site of injury (15). CD40 ligation on endothelial cells, VSMCs, and macrophages initiates the expression and release of matrix metalloproteinases (MMPs) (16). One of them, MMP-2, was identified by Fernandez-Patron et al. in human platelets (17). The release of MMP-2 during platelet activation mediates aggregation in a non-thromboxane-, non-ADP-dependent manner. Along with the characterisation of MMP-2, MMP-9 was identified to be an inhibitor of platelet aggregation suggesting that the MMP-9/MMP-2-dependent bioregulatory system might be involved in platelet-platelet and platelet-vessel wall interactions (17). Moreover, platelet adhesion via glycoprotein (GP) IIb/IIIa upregulates CD40L and P-selectin (CD62P) surface exposure leading to the assumption, that clinical benefits of GP IIb/IIIa antagonists in addition to the inhibition of thrombosis by blocking platelet aggregation also involve the inhibition of inflammation and thrombosis via blockade of CD40L release (18, 19). While molecular interactions of leukocytes with activated endothelial cells are critical for the defense against microbial invasion and immune trafficking, platelets also contribute to leucocyte targeting- and regulated accumulation (20–22). The role of platelet-leucocyte aggregates (PLA) in cardiovascular disease will be discussed in another chapter of this Theme Issue.

Table 1: Selection of inflammatory and immune modulating factors and mediators released by activated human platelets and/or translocated to their plasma membranes.

<table>
<thead>
<tr>
<th>Class of mediator</th>
<th>Factor</th>
<th>Target cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleiotropic inflammatory and immune modulators</td>
<td>Histamine</td>
<td>ECs, monocytes, neutrophils, NK cells, T- and B cells, eosinophils</td>
</tr>
<tr>
<td></td>
<td>Serotonin (5-HT)</td>
<td>Monocytes, macrophages, DCs</td>
</tr>
<tr>
<td>Inflammatory and immunomodulatory lipids</td>
<td>TBXA2</td>
<td>Platelets, T-lymphocytes, macrophage subsets</td>
</tr>
<tr>
<td></td>
<td>PAF</td>
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</tr>
<tr>
<td>Growth factors with immune activities</td>
<td>TGF-β</td>
<td>Monocytes, macrophages, T and B lymphocytes</td>
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<tr>
<td></td>
<td>PDGF</td>
<td>Monocytes, macrophages, T lymphocytes</td>
</tr>
<tr>
<td>Chemokines</td>
<td>NAP2 (CXCL7)</td>
<td>Neutrophils</td>
</tr>
<tr>
<td></td>
<td>PF4 (CXCL4)</td>
<td>Neutrophils, monocytes, macrophages</td>
</tr>
<tr>
<td></td>
<td>GRO-α (CXCL1)</td>
<td>Neutrophils</td>
</tr>
<tr>
<td></td>
<td>ENA-78 (CXCL5)</td>
<td>Neutrophils</td>
</tr>
<tr>
<td></td>
<td>SDF-1 (CXCL12)</td>
<td>Bone marrow-derived progenitor cells</td>
</tr>
<tr>
<td></td>
<td>RANTES (CCL5)</td>
<td>Monocytes, eosinophil, basophil, NK cells, T-lymphocytes, DC subsets</td>
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<tr>
<td></td>
<td>MIP-1α (CCL3)</td>
<td>Monocytes, eosinophils, basophil, NK cells, lymphocytes, DC subsets</td>
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<tr>
<td></td>
<td>MCP-3 (CCL7)</td>
<td>Monocytes, basophil, NK cells, lymphocytes, DC subsets</td>
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<tr>
<td></td>
<td>MIF</td>
<td>Monocytes, macrophages</td>
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<tr>
<td>Cytokines</td>
<td>CD40L (CD154)</td>
<td>ECs, monocytes, DC subsets, lymphocyte subtypes</td>
</tr>
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<td></td>
<td>IL-1β</td>
<td>Monocytes, DC and macrophage subsets, T cells, ECs, VSMCs, synoviocytes</td>
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<td>IL-1α</td>
<td>Monocytes, DC and macrophage subsets, T cells, ECs, VSMCs, synoviocytes</td>
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<td></td>
<td>HMBG1</td>
<td>Macrophages, neutrophils, ECs</td>
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<td>GM-CSF</td>
<td>Neutrophils, eosinophil, monocytes, macrophages</td>
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<tr>
<td>Cell adhesion molecules and integrins</td>
<td>P-Selectin (CD62P)</td>
<td>ECs, neutrophils, eosinophils, monocytes via PSGL-1</td>
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<td></td>
<td>GPⅡb/Ⅲa</td>
<td>Neutrophils, monocytes</td>
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Selected novel platelet-derived targets involved in inflammatory pathways in CAD

Stromal cell derived factor-1α (SDF-1α) and receptors CXCR4-CXCR7

Platelet activation through ADP-receptor (P2Y12), GPVI and protease activated receptor-1 (PAR1) ligation drives SDF-1α (CXCL12) release (23) (Figure 1) and enhances migration and differentiation of endothelial progenitor cells at the sites of vascular injury to promote regeneration (24, 25). Platelet-derived SDF-1α also promotes the differentiation of CD34+ cells into macrophages and foam cells and thereby contributes to vascular inflammation (26). CXCR4 and CXCR7 are the G protein-coupled receptors (GPCRs) designated for SDF-1α (27). SDF-1α, although considered a weak agonist, stimulates platelet migration (28, 29). Both chemokine receptors CXCR4-CXCR7 have been characterised in human (30) and murine (31) platelets, which show a dynamic bidirectional trafficking in response to SDF-1α and Macrophage migration inhibitory factor (MIF), thereby influencing relative receptor availability on the platelet surface (31). Both, SDF-1α and MIF induce CXCR4 internalisation, which is coupled to CXCR7 surface translocation only under the influence of SDF-1α. SDF-1α/CXCR4-triggered CXCR7 exposure is mediated through Erk1/2 phosphorylation and intracellular Cyclophilin A (CypA)-PPIase activity resulting in CXCR7 ubiquitination, and thereby its translocation to platelet surface (Figure 1) (31). Unlike SDF-1α, MIF-CXCR4 ligation does not lead to Erk1/2 activation in platelets and T cells due to the absence of co-receptor CD74. Therefore, MIF fails in subsequent CXCR7 surface translocation. This suggests a ligand-specific (MIF vs SDF-1α) effect on CXCR4-triggered signalling in platelets, possibly to govern distinct cellular functions (32). Increased surface expression of CXCR7 among patients with acute coronary syndrome (ACS) correlates with the expression of its ligand SDF-1α and recovery of the ventricular function (30). These findings indicate that platelet surface expression of CXCR4 and CXCR7 might differentially contribute to SDF-1α mediated regenerative mechanisms, which warrant further evaluation regarding the mechanistic basis of CXCR4/7 expression and its prognostic impact in coronary artery disease (CAD).

Macrophage Migration Inhibitory Factor (MIF)

MIF is designated as a pleiotropic chemokine like cytokine and contributes to vascular inflammation, atheropresentation, plaque vulnerability, sepsis, rheumatoid arthritis, inflammatory and autoimmune diseases (33). Platelets harbour three fometag MIF/cell and – owing to their abundance in circulation - might have a profound influence in modulating vascular inflammation by instigating monocyte migration (34) or potentially contributing to elevated plasma MIF levels in patients with acute myocardial infarction (MI) (35). MIF rescues platelets from undergoing apoptosis through CXCR7 like SDF-1α and CXCL11 and sustains platelet life span in circulation as well (32). Since the anti-apoptotic effect of the MIF/CXCR7 interaction involves an attenuation of prothrombotic phospholipaseA2 exposure on the platelet surface, MIF counter-regulates thrombus formation, both in vitro and following arterial injury in vivo through CXCR7 involvement without altering integrin activation (Pancaspase Activating Compound-1 [PAC-1] binding) (32). This could bear promising clinical relevance in patients undergoing anti-platelet therapy against cardiovascular syndromes and cerebral ischaemia. MIF could potentially emerge as a relevant physiological candidate that checks thrombotic aspects without compromising the haemostatic and regenerative capacity of platelets. Moreover, MIF might foster platelet survival to ensure sustained platelet-dependent regenerative mechanisms. Platelets represent a source of chemokines, cytokines, and growth factors, which modulate platelet-leucocyte or platelet-endothelial cell interactions. They show sustained survival in blood circulation, which may profoundly influence the duration and resolution of inflammatory processes, e.g. onset and progression of atherosclerosis, plaque stability and CAD.

Figure 1: The SDF-1α-MIF interplay in regulating CXCR4-CXCR7 availability, platelet function and survival. A) Representative immunofluorescence confocal microscopic images (n=5) showing intracellular localisation of MIF, SDF-1α and CXCL11 (Alexa Fluor 488-green) in a granular randomly distributed pattern across platelet cytoplasm (Bar=2µm) (32). B) Bar diagram of the flow cytometry data representing release of MIF, SDF-1α and CXCL11 denoted by their relative surface expression with respect to resting platelets, upon activation with ADP (100 µM), PAR-1 activator TRAP (25 µM), PAR-4 activating peptide (100µM) and CRP (10µg/ml). *P<0.05 as compared to resting platelets. Data presented as mean±S.E.M of five independent experiments (32). C) Schematic representation of the surface receptors for MIF, SDF-1α and CXCL11 on platelet surface. D) Schematic representation demonstrating CXCR4 ligation with SDF-1α induces internalisation of CXCR4 and externalisation of CXCR7. Enhanced surface exposure of CXCR7 upon SDF-1α-CXCR4 ligation is mediated through Erk1/2 activation (inhibited in presence of U0126) and involvement of CyPA-PPIase activity (inhibited by NIM-811), enhancing CXCR7 ubiquitination (counteracted by E1li-gase inhibitor PYR41). Ubiquitinated CXCR7 is translocated to platelet surface. SDF-1α provides pro-survival benefit to platelets against activation induced apoptosis as more CXCR7 is available on the surface for subsequent SDF-1α ligation. MIF also binds to CXCR4 and internalises the receptor in platelets. However, due to the absence of the co-receptor CD74 on platelet surface, MIF cannot transduce signals through CXCR4 to Erk. Therefore, MIF binds directly to the CXCR7 available on platelets and promotes survival against activation induced apoptosis. MIF induces downstream phosphorylation of PI3K-Akt and the phosphorylation mediated inactivation of proapoptotic protein BAD (Bcl2 antagonist of cell death) to exert its prosurvival effects (32). Methods: Human platelets were either kept untreated (for basal level of MIF/CXCL11 surface expression) or treated with rhMIF (200 ng/ml) or rhCXCL11 (100 ng/ml) for 10 min at RT in presence/absence of blocking antibodies against CXCR4-CXCR7 (10µg/ml), or respective IgG controls (10 µg/ml) as indicated. Platelets were treated with blocking antibodies/IgG controls for 30 mins at RT before addition of rhMIF/rhCXCL11. Thereafter, cells were treated with anti-MIF-FITC or anti-CXCL11-FITC antibody for 30 mins at RT, fixed in 0.5% paraformaldehyde and analysed for the surface binding of MIF/CXCL11 by flow cytometry.
Cyclophilin A (CypA)

CypA serves as a molecular chaperone and functions as a redox stress sensitive proinflammatory cytokine that contributes to cardiac hypertrophy, atherosclerosis, MI, and myocarditis (36). CypA-PPIase activity participates in the intracellular bidirectional trafficking of CXCR7 following SDF1α-CXCR4 ligation. CypA interacts with phosphorylated-Erk1/2 downstream of CXCR4.
ligation. Furthermore, CypA-PPase facilitates ubiquitination of CXCR7 translocating it to the platelet surface (31). CypA is also involved in influencing thrombosis and haemostasis functions of platelets as elaborated in CypA+/− mice (37, 38). CypA functions as a critical calcium sensor and regulator for store release and refill as mediated by Orai1 (38). CypA-dependent increase in ROS production leads to inside-out signalling causing increased GP IIb/IIIa activation and enhanced binding to fibrinogen. CypA has a dual effect in regulating bidirectional signalling as mediated through platelet GP IIb/IIIa (37). On the other hand platelet-derived CypA might have potential immune-regulatory and inflammatory consequences, when released following platelet activation and execute multiple effector mechanisms via the CypA receptor EMMPRIN (CD147), on its target cells like endothelial cells, monocytes, and macrophages leading to aggravation of proinflammatory signalling cascades. Rigorous and advanced proteomic analysis of activated platelet releasate identified CypA as one of > 300 proteins. CypA was localised in atherosclerotic plaque regions, where platelets accumulate (39). Therefore, future studies should be directed towards deciphering the potential pathophysiological implications of platelet derived CypA in exerting both, autocrine (outside in signalling) and paracrine effects on target cells.

C-reactive protein (CRP)

CRP is a highly conserved protein of the pentaxin family consisting of five non-covalently linked subunits of 23 kDa (40). CRP is mainly produced in the liver but can also be secreted by endothelial and smooth muscle cells (41). Several prospective clinical studies have shown independently that modest elevations in baseline CRP levels, as detected by high-sensitivity assays, predict future cardiovascular events (42–45). There is emerging evidence of CRP as a direct and causative factor contributing to development of atherosclerosis (46, 47). CRP increases monocyte adhesion to human endothelial cells under flow conditions (48) and is released from vulnerable plaques, where it co-localises with macrophages, oxLDL, and complement factors (49–51). In human plasma, CRP exists as a cyclic, disc-shaped pentamer of 115 kDa (pentameric pCRP) (40) or as a monomeric or modified mCRP (52, 53). At higher concentrations, pCRP evolves proinflammatory properties and has been associated with chronic diseases such as hypertension, metabolic syndrome and type 2 diabetes (54, 55). In contrast, low concentration mCRP induces interleukin-8 (IL-8) secretion by neutrophils and human coronary artery endothelial cells, promotes monocyte migration, neutrophil-endothelial cell adhesion, and delays apoptosis of human neutrophils (53, 56, 57) along with different interactions with the complement cascade (58). Recently, it was shown that mCRP is present in atherosclerotic lesions of human aorta and carotid arteries. Activated platelets found at atherosclerotic lesions can dissociate native pCRP into mCRP. Khreiss et al. have shown a shear-induced upregulation of platelet CD62P expression under mCRP, leading to neutrophil-adhesion on platelets and subsequent neutrophil aggregation (59). Today, little is known about the in vivo effects of mCRP, partially due to lack of suitable animal models. In this context, Teupser et al. could show that CRP-deficient mice developed atherosclerosis to the same degree as the control group (60). Neither the reduction of CRP serum concentration nor its complete absence resulted in a significant reduction of atherosclerotic lesions in the brachiocephalic arteries and aortic roots in two distinct mouse models of atherogenesis including a morphometric analysis of 240 animals. In two CRP+/− subgroups, Teupser et al. observed an increase in aortic root lesions suggesting an antiatherogenic (not proatherogenic) influence of CRP in mice. These findings question proposed strategies aimed at atheroprosession by pharmaceutical intervention to reduce CRP serum levels or CRP activity. Still, no currently available animal model of atherosclerosis can fully reflect atherogenesis and atheroprogession in humans, as there are interspecies differences. For example, in mice, the major acute phase protein is the homologous pentraxin serum amyloid protein, whereas CRP plasma levels rise only moderately following appropriate stimulation. Therefore, extrapolation of data from mouse to human is to be treated with caution.

**Platelet microRNA**

The impact of platelet messengerRNA (mRNA) and microRNA (miRNA) is still a matter of debate. Platelets do not contain a nucleus and therefore, regulatory effects of coding and non-coding RNA remain controversial. mRNA analysis and transcriptomics as well as non-coding RNA are rapidly evolving fields of research, which provide crucial insights regarding age, gender and demographic variants that determine differences in platelet hyper-reactivity (61, 62). Recent evidence suggests that miRNA containing microparticles derived from platelets are involved in regulation of gene expression in endothelial cells by means of functional Argonaute 2 (Ago2) mRNA complexes (63). Inter- and intraindividual discrepancies could account for the variant response to antiplatelet treatments (e.g. clopidogrel hyporesponsiveness), help to identify patients at risk for cardiovascular diseases, and optimise their subsequent risk management. Individuals exhibiting platelet hyper-reactivity are at thrombotic risk, which might lead to MI, stroke and peripheral artery disease (PAD), whereas platelet hypo-reactivity predisposes to haemorrhagic disorders. Comparison of distinct platelet transcriptomes reveals that hyper-reactive platelets express up to five-fold higher mRNA levels than hypo-reactive platelets. Apart from spliceosomes that regulate the synthesis of IL-1b in activated platelets through pre-mRNA splicing, platelets also contain small (around 23 base pair) conserved and non-coding miRNAs derived from hairpin-like precursors, which can specifically silence their target miRNAs thereby participating in post-transcriptional gene regulation. Moreover detection of pre-miRNA transcripts for some abundant platelet miRNAs (miR-223, let-7c and miR-19a) and presence of core components of miRNA effector complex, i.e. Dicer and Ago2, suggests biogenesis of mature miRNA from pre-miRNA templates. Recently, reports from the platelet RNA and expression-1 study have been published which measured platelet aggregation response to arachidonic acid (AA), ADP, PAR1-PAR4 stimulation, and corresponding mRNA and miRNA levels in platelets from 154 healthy subjects of different...
ethnicity. Data of this extensive cohort reveal differential expression of 129 mRNAs and 15 miRNAs by age, 54 mRNAs and 9 miRNAs by gender highlighting the inverse relationship between these mRNAs and miRNAs. An interactive web tool has been generated (www.plateletomics.com) which permits queries regarding RNA levels and associations among RNA, platelet aggregation and demographic variables (61). Platelet miRNAs bind to important target mRNAs that govern P2Y12, GPIIb/IIIa, and cyclic AMP-dependent protein kinase A. The role of miR-34a and miR-150 has been implicated in megakaryocytopoiesis and platelet biogenesis, whereas mature platelets are known to harbour 284 miRNAs: the most abundant is miR-223 followed by miR-126. miR-96, miR-15a, miR-339–3p, miR-365, miR-495, miR-98, and miR-361–3p show differential expression upon thrombin stimulation (67). Further insights into the miRNA profile of circulating platelets regulating their life span as part of physiological platelet turnover during inflammation, immune or drug-induced thrombocytopenia would be particularly welcome. Moreover, platelet-derived micro-vesicles/particles contain several miRNAs, which might fine-tune potential pathophysiological interactions between platelets with other vascular cells in the process of maintaining vascular homeostasis. Platelet miRNAs can contribute to plasma pools and potentially serve as biomarkers (62). Levels of miR-340* and miR-624* are significantly high in platelets from patients affected with premature CAD (68). Further, the possibility of manipulating platelet miRNAs expression and function as therapeutic tool can bear significant effects on specific protein levels and overall platelet reactivity.

Figure 2: Platelet activation and aggregation pathways, inhibition sites of antiplatelet drugs. Platelet activation via multiple pathways leads to various responses including shape change, dense granule secretion of ATP, 5-Hydroxytryptamin (5-HT, serotonin), and ADP (which binds to P2Y1 and P2Y12 receptors, playing a pivotal role in activation amplification), α granule secretion of chemokines leading to leucocyte and endothelial cell activation, and coagulation factors enhancing thrombin generation and activation of GPIIb/IIIa leading to platelet aggregation and further platelet activation. Adapted with permission from Storey (70) and Patrono et al. (71).
Clinical impact of platelet-mediated inflammation and anti-inflammatory effects of antiplatelet therapy in cardiovascular disease

Pathophysiology of platelet activation and aggregation

Platelet activation and aggregation play a pivotal role in atherogenesis and atheroprogession. Platelet- and thrombus formation is initiated by the adherence of platelets to subendothelial collagen via von Willebrand factor (VWF) and the GP receptor complex at the site of vessel wall injury (69). This leads to an activation of phospholipase C (PLC)-mediated cascade resulting in calcium release from the dense tubular system. An increase in intracellular calcium activates several kinases necessary for the secretion of platelet granular content, the activation of GPs, and the activation of phospholipase A2 (PLA2) preceding AA release, a precursor of thromboxane A2 (TXA2). This pathway is irreversibly blocked by acetylsalicylic acid (ASA) via COX-1 inhibition and represents a major target to inhibit platelet aggregation. The activation processes result in further formation and accumulation of prothrombotic molecules including thrombin, TXA2, and ADP, which maintain platelet activation mediated by their respective G protein coupled receptors (thrombin receptor [F2R], thromboxane A2 receptor [TXA2R], and ADP receptors [P2Y1 and P2Y12]). When activated by ADP, the P2Y12 pathway inhibits adenylate cyclase. Thienopyridines, a class of oral antiplatelet agents, inhibit P2Y12 signalling, thus effectively blocking ADP-dependent platelet activation (70, 71).

Platelet adhesion, cytoskeletal reorganisation, secretion, and amplification loops are all important but different steps towards the formation of a thrombus. These respective signalling cascades result in the activation of the fibrinogen receptor expressed on platelets. Binding sites for fibrinogen are only found in activated platelets leading to clotting of activated platelets via fibrinogen bridges, thereby mediating aggregation. Inhibition of this receptor via GPIIb/IIIa inhibitors blocks platelet aggregation induced by any agonist of this pathway. Platelet activation and aggregation along with established targets of platelet inhibition are illustrated in Figure 2.

Furthermore, activated platelets induce tissue factor (TF) expression in human endothelial and smooth muscle cells, presumably by releasing soluble mediators such as serotonin and PDGF (72). Platelets also synthesise plasminogen activator inhibitor (PAI)-1, a main regulator of fibrinolysis (73).

The previously described PLA are formed after platelet activation and degranulation. Activated platelets preferentially engage with monocytes via platelet CD62P, CD42b (glycoprotein IB, GPIB) and junctional adhesion molecule C (JAM-C) binding to the monocyte receptors P-selectin glycoprotein ligand-1 (PSGL-1) (74) and Macrophage-1 antigen (MAC-1, monocyte CD11b). Platelet-monocyte aggregates induce TF, proinflammatory factors and the production of microparticles from both cell types (75). Platelet CD62P also stimulates monocytes to synthesise platelet-activating factor, thus leading to further progressive and uncontrolled platelet activation (76).

Hence, platelets play an important amplifying role in inflammation, vascular injury and atherothrombosis. Antiplatelet strategies therefore are expected to impact on platelet-derived protein signals for the procoagulant and inflammatory response (71, 77, 78).

Correlation between platelet aggregation and inflammatory markers in patients on antiplatelet therapy

Several studies have linked platelet aggregation measured by different agonist induced pathways and inflammatory marker levels. In a recent study, high platelet reactivity (PR) measured by ADP induced light transmission aggregometry (LTA) was associated with hs-CRP levels, soluble CD62P and CD40L (79).

We found that ADP induced platelet aggregation was significantly associated with CRP-levels and IL-6 levels, whereas AA induced PR correlated with CRP, IL-6 and RANTES levels. In addition, there were complementary effects of CRP and ADP-induced PR to predict stent thrombosis in patients with CAD undergoing percutaneous coronary intervention (PCI) (54).

Furthermore, Antonino et al. found a strong correlation between platelet aggregation and specific inflammatory markers in patients undergoing clopidogrel treatment (80). Since antiplatelet strategies impact on both the procoagulant and inflammatory response of platelets, it is important to be aware of the large variety of antiplatelet agents that affect different pathways of platelet activation (Figure 2, Table 2).

Anti-inflammatory effects of antiplatelet agents

Acetylic salicylic acid (ASA)

ASA binds irreversibly to cyclooxgenase (COX)-1 and –2 and inhibits the production of TXB2. This is the key mechanism of

Table 2: Available antiplatelet agents for clinical use and clinical research.

<table>
<thead>
<tr>
<th>Class of antiplatelet agent</th>
<th>Antiplatelet agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX-1 inhibitors</td>
<td>Irreversible: ASA</td>
</tr>
<tr>
<td></td>
<td>Reversible: indobufen, triflusal</td>
</tr>
<tr>
<td>P2Y12 inhibitors</td>
<td>Irreversible: ticlopidine, clopidogrel, prasugrel</td>
</tr>
<tr>
<td></td>
<td>Reversible: ticagrelor, cangrelor, elinogrel</td>
</tr>
<tr>
<td>GPIIb/IIIa inhibitors</td>
<td>Abciximab, eptifibatide, tirofiban</td>
</tr>
<tr>
<td>Thrombin receptor (PAR-1) antagonists</td>
<td>Vorapaxar, atopaxar</td>
</tr>
<tr>
<td>Thromboxane receptor antagonists</td>
<td>Terutroban</td>
</tr>
<tr>
<td>Phosphodiesterase inhibitors</td>
<td>Cilostazol, dipyridamole</td>
</tr>
<tr>
<td>ASA -- acetylsalicylic acid (aspirin); COX-1 -- cyclooxygenase-1; PAR-1 -- Protease-activated receptor; P2Y12 -- ADP receptor.</td>
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ASA as an inhibitor of platelet aggregation and vasoconstriction, which are both TBXA2 induced.

ASA is unique in not only promoting analgesic, antipyretic, and anti-inflammatory effects, but it also shows beneficial impact on the cardiovascular system via anti-inflammatory pathways distinct from prostaglandin and TBXA2 inhibition along with anti-aggregatory functions. These effects are depending on the dose of ASA that is applied.

COX-1 is present in all tissues. In healthy people, 95% suppression of platelet COX-1 activity is achieved with low-dose ASA. COX-1 produces prostaglandins mainly involved in physiological processes and house keeping functions, like platelet aggregation, homeostasis, gastric epithelial cytoprotection and also inflammation. The COX-2 isofrom is especially expressed in proliferative diseases and inflammatory states in response to cytokines or acute phase proteins and can be found in atherosclerotic plaques, newly formed platelets and also induces prostacyclin synthesis. In contrast to COX-1, higher doses of ASA are needed to block COX-2 and to obtain anti-inflammatory effects. Low doses of ASA achieve adequate antiplatelet effects but not sufficient anti-inflammatory effects.

Another anti-inflammatory mechanism of ASA is mediated via nuclear factor (NF)-κB-dependent induction of adhesion molecules in endothelial cells and monocytes.

However, these mechanisms alone do not explain all of the effects that are described for ASA, in particular, its ability to limit leukocyte migration to the site of inflammation and thus to successfully attenuate the inflammatory response.

ASA is an important inhibitor of platelet activation via TBXA2, but also reduces endothelial cell production of prostacyclin. Endothelial cells regenerate active COX faster than platelets, because mature platelets cannot synthesise the enzyme, thus requiring new platelets to enter the circulation. Therefore, the net effect of ASA is more in favour of endothelial cell-mediated inhibition of the coagulation cascade (82).

Part of ASAs cardiovascular benefits is related to its dose-dependent differential effects on inflammatory processes. The low dose anti-inflammatory impact of ASA (e.g. 81 or 100 mg) is ascribed to its ability to trigger the synthesis of the lipoxins (LXs), especially LXA4 and LXB4 (83). Higher doses of aspirin have no significant effect on LX synthesis. The lipoxins are anti-inflammatory eicosanoids synthesised through lipoxigenase interactions. The anti-inflammatory actions of the LXs are explained by their ability to inhibit the actions of the leukotrienes (83–85).

Lipoxin synthesis from AA occurs via several different transcellular interactions, amongst others by involving 5-lipoxygenase (5-LOX) activity in leukocytes followed by 12-LOX action in platelets. The action of 15-LOX in epithelial cells followed by 5-LOX action in leukocytes is the second major LX synthesis pathway (86).

It has been described, that ASA inhibits the action of COX-1 and COX-2 by acetylation; however, in endothelial and epithelial cells the effect of ASA on COX-2 alters the enzyme such that it now converts AA to 15R hydroxyeicosatetraenoic acid. This compound is then rapidly metabolised to LXs in monocytes and leukocytes via the action of 5-LOX, a consequence requiring, that platelets interact directly with adherent neutrophils/monocytes. There is also an ASA-triggered pathway leading to LX synthesis. This mechanism is initiated, when activated circulating leukocytes (primarily neutrophils) adhere to the vascular endothelium (83, 86, 87).

Additional anti-inflammatory actions of LXs and ASA-triggered LXs include blocking the expression of the IL-8 gene, a pro-inflammatory chemokine produced by macrophages and endothelial cells that stimulates neutrophil migration. Furthermore, TNF-α release is inhibited (87, 88). In conclusion, the anti-inflammatory effects of ASA are mediated by dose-dependent, combinatorial effects on platelet- and leukocyte inflammatory responses.

There is a growing body of evidence from clinical studies that the anti-inflammatory effect of ASA is of clinical relevance regarding inflammatory cytokines and CRP levels in cardiovascular patients. In the Physician’s Health Study (n=1086), CRP was an independent predictor of MI and stroke in apparently healthy men at baseline. ASA significantly reduced the risk of MI by 44% (p< 0.00001) (89). The benefits of ASA increase linearly with CRP levels and cardiovascular risk. For instance, patients with the highest baseline CRP levels derived the greatest benefit from ASA therapy (89). Another trial had to be stopped prematurely, as ASA had a significant risk reducing effect on the occurrence of the first MI (90). There is remarkable evidence that low-dose ASA is of substantial net benefit for patients who already have occlusive vascular disease. Its benefits in primary prevention were shown in meta-analyses by Baigent et al. (91) and Bartolucci et al. (92). These findings were put into perspective in recent reviews (93–95). Serious vascular events (MI, stroke, or cardiovascular death) and major bleeds in six primary prevention large-scale trials (95,000 individuals at low average risk, 660,000 person-years, 3,554 serious vascular events) were analysed. In the primary prevention trials, ASA was associated with a 12% proportional reduction in serious vascular events (0.51% aspirin vs 0.57% control per year, p=0.0001), mainly due to a reduction of about a fifth in non-fatal MI (0.18% vs 0.23% per year, p<0.0001), but at the cost of an increase of 54% in major extracranial bleeding including major gastrointestinal bleeds (0.10% vs 0.07% per year, p<0.0001), regardless of the baseline risk (91-93). Thus, the net clinical benefits of low dose ASA have to be regarded in the context of baseline risk and net clinical benefit, the latter is diminished in low risk primary prevention cohorts. Primary prevention with ASA is widely applied, however, not only because of its cardioprotective impact but also because there is increasing evidence of chemoprotection by ASA against cancer (96). Regarding its effect on the inflammatory response, the reduction of CRP levels by ASA has also been confirmed in other patient cohorts including subgroups with stable CAD (97) and ACS (98, 99). Furthermore, ASA treatment reduces levels of IL-6, MCP-1, transforming growth factor-β (TGF-β), and TNF-α (97–100) but does not affect platelet-leukocyte interactions (101) (Table 3). Of note, in these studies ASA was given in doses, which are not considered to have direct anti-inflammatory effects, thus the influence on the inflammatory markers might be due to indirect effects caused by platelet inhibition. The modest reduction in cytokine levels might have been related to the low dose...
used in the study, as ASA shows the greatest anti-inflammatory effect at doses higher than 1.2 g.

**Clopidogrel**

The P2Y12 receptor antagonist clopidogrel, a second generation thienopyridine, selectively and irreversibly inhibits the ADP receptor on platelets, thereby blocking agonist-induced platelet aggregation. The ADP receptor has also been linked to other processes, including fibrinogen receptor activation and CD62P exposure (102). Like ASA, clopidogrel reduces the expression of inflammatory markers in patients with cardiovascular disease, but there are no direct mechanisms detected so far. That implies even more that the anti-inflammatory effect and its clinical benefit are caused by its anti-platelet activity. By inhibiting platelet activation, along with CD62P expression, clopidogrel can interfere with leukocyte-platelet interactions and the consequent increase in inflammatory markers that take place during the thrombotic process (103, 104).

### Table 3: Influence of antiplatelet agents on inflammatory markers in primary and secondary prevention cardiovascular trials.

<table>
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<tr>
<th>Study design and study population</th>
<th>Condition / Disease state</th>
<th>Antplatelet agents</th>
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<tr>
<td><strong>ASA monotherapy</strong></td>
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<tr>
<td>Prospective sub-study of the randomised, double-blind Physician’s Health Study (89)</td>
<td>Healthy men who developed MI, stroke, or venous thrombosis during the study (n=543)</td>
<td>ASA vs Placebo</td>
<td>CRP</td>
<td>ASA significantly reduced the risk of MI, increasing benefit with increasing plasma CRP levels (p=0.02)</td>
</tr>
<tr>
<td>Prospective sub-study of the randomised, double-blind Physician’s Health Study (89)</td>
<td>Healthy men who did not develop vascular disease during the study (n=543)</td>
<td>ASA vs Placebo</td>
<td>CRP, M-CSF, IL-6, IL-1β</td>
<td>Plasma CRP, IL-6, and M-CSF levels significantly reduced in ASA arm (P&lt;0.05)</td>
</tr>
<tr>
<td>Prospective, randomised crossover study (97)</td>
<td>Stable CAD</td>
<td>ASA vs Placebo</td>
<td>CRP, M-CSF, IL-6, IL-1β</td>
<td>hsCRP levels and TNF-α significantly lower in ASA-treated arms at 4 years (p=0.023 and p=0.034, respectively)</td>
</tr>
<tr>
<td>Prospective study of the randomised, open-design WARIS II trial (99)</td>
<td>ACS</td>
<td>ASA vs ASA and Warfarin vs Warfarin</td>
<td>hsCRP, TNF-α, IL-6, IL-10</td>
<td>hsCRP levels and TNF-α significantly lower in ASA-treated arms at 4 years (p=0.023 and p=0.034, respectively)</td>
</tr>
<tr>
<td>Prospective sub-study of the randomised ASCET trial (100)</td>
<td>Stable CAD</td>
<td>ASA vs Clopidogrel</td>
<td>hsCRP, TNF-α, IL-6, IL-10, MCP-1, CD62P, TGF-β, CD40L</td>
<td>No significant differences in ASA vs Clopidogrel arm at 1 month and 1 year</td>
</tr>
<tr>
<td><strong>Clopidogrel monotherapy</strong></td>
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<tr>
<td>Prospective, non-randomised study (105)</td>
<td>Healthy volunteers (n=10)</td>
<td>Healthy population</td>
<td>Clopidogrel</td>
<td>PLA, CD62P</td>
</tr>
<tr>
<td>Prospective, non-randomised study (110)</td>
<td>Type 2 diabetes mellitus (n=20) without clinical evidence of CAD or chronic inflammatory disease</td>
<td>Clopidogrel</td>
<td>PLA, RANTES, CD40L, sCD40L, sE-selectin, monocyte CD40 and CD11b</td>
<td>Reduction of platelet CD62P (p=0.002) but not CD40L expression Monocyte surface expression of CD40 (p=0.007) and CD11b (p=0.02) were reduced after clopidogrel treatment. Reduced platelet–monocyte (p=0.01) and platelet–neutrophil (p=0.04) aggregates (PLA) Decrease of RANTES (p&lt;0.0001) after clopidogrel treatment.</td>
</tr>
<tr>
<td>Prospective sub-study of the randomised ASCET trial (100)</td>
<td>Stable CAD</td>
<td>ASA vs Clopidogrel</td>
<td>hsCRP, TNF-α, IL-6, IL-10, MCP-1, CD62P, TGF-β, CD40L</td>
<td>No significant differences in ASA vs Clopidogrel arm at 1 month and 1 year</td>
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Table 3: Continued

<table>
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<tr>
<th>Study design and study population</th>
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<tr>
<td><strong>Dual antiplatelet therapy with ASA and clopidogrel</strong></td>
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<tr>
<td>Prospective, non-randomised study (109) PCI patients (n=79, n=33 pretreated with clopidogrel, n=46 not pretreated with clopidogrel) Duration: 24 h</td>
<td>CAD patients undergoing PCI</td>
<td>Pretreatment with Clopidogrel(^1) vs No Clopidogrel(^1)</td>
<td>CD40L, CD62P, sCD40L, IL-6</td>
<td>Platelet CD40L and CD62P levels significantly lower at baseline and post-PCI (CD40L: p=0.002 and p=0.0007, respectively, CD62P: p&lt;0.001 and p=0.001, respectively) in clopidogrel arm Clopidogrel pretreatment did not affect serum IL-6 or sCD40L levels</td>
</tr>
<tr>
<td>Prospective randomised study (121) (PROCLAIM) Patients with metabolic syndrome received clopidogrel 75 mg/day + ASA 81 mg/day (n=89) or placebo + ASA aspirin 81 mg/day (n=92) Duration: 6 to 9 weeks</td>
<td>Patients with metabolic syndrome</td>
<td>ASA+clopidogrel vs ASA+placebo</td>
<td>hsCRP, CD40L, CD62P</td>
<td>Significant decrease of CD40L levels at week 6 in the ASA+clopidogrel arm compared with ASA+placebo (p=0.02)</td>
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<tr>
<td>Prospective, non-randomised study (80) 110 consecutive patients (clopidogrel-naive patients, n=69, and patients receiving long-term clopidogrel therapy for &gt;6 months, n=41) before nonemergent PCI Duration: 24 h</td>
<td>Stable CAD undergoing PCI</td>
<td>ASA+Clopidogrel</td>
<td>CD62P, TF-PCA, GPIIb/IIa</td>
<td>Pre-PCI expression of CD62P and active GP IIb/IIa was lower in patients on long-term clopidogrel therapy compared with the clopidogrel-naive group (p&lt;0.001) Lower levels of CD62P, TF-PCA (p&lt; or =0.05)</td>
</tr>
<tr>
<td>Prospective, non-randomised study (106) Patients with NSTEMI (n=23) Duration: 24 h</td>
<td>ACS</td>
<td>Clopidogrel LD2 (300 mg)</td>
<td>PLA, CD62P, sCD40L, sCD62P</td>
<td>Significant reduction of ADP- and TRAP-induced CD62P levels by clopidogrel (p&lt;0.01) Significant decrease of sCD40L and sCD62P by clopidogrel (p&lt;0.001) PLA formation significantly reduced by clopidogrel (p&lt;0.01)</td>
</tr>
<tr>
<td>Prospective, non-randomised study of PCI registry (183) Consecutive PCI patients (n=833) Duration: 5 days</td>
<td>Patients undergoing PCI</td>
<td>Clopidogrel LD3 (300–600 mg)</td>
<td>hsCRP</td>
<td>Periprocedural increase of hsCRP was significantly reduced by clopidogrel (p=0.03)</td>
</tr>
<tr>
<td>Randomised, double-blind, parallel group study (ALBION) (184) NSTEMI patients (n=103) Duration: 24 h</td>
<td>ACS</td>
<td>Clopidogrel LD4 (300 mg vs 600 mg vs 900 mg)</td>
<td>hsCRP, sCD40L, PAI-1, and vWF</td>
<td>No significant differences between treatment arms</td>
</tr>
<tr>
<td>Prospective, randomised, double-blind study (98) Patients with NSTEMI (n=115) Duration: 30 days</td>
<td>ACS</td>
<td>ASA vs ASA+Clopidogrel</td>
<td>hsCRP, TNF-α</td>
<td>At day 7 and day 30, significantly reduced levels of hsCRP and TNF-α in ASA and ASA+Clopidogrel arm compared to control (p&lt;0.01) At day 7 and day 30 hsCRP was significantly lower in ASA+Clopidogrel arm (p&lt;0.05) At day 30 TNF-α was significantly lower in ASA+Clopidogrel arm (p&lt;0.05)</td>
</tr>
<tr>
<td>Prospective, randomised, single-blind study (108) Patients with NSTEMI (n=86) Duration: 36 weeks</td>
<td>ACS</td>
<td>ASA+Clopidogrel(^4) vs ASA(^4)</td>
<td>sCD62P, sCD40L, hsCRP</td>
<td>Mean sCD62P levels significantly lower in ASA+Clopidogrel arm, that showed elevated hsCRP and sCD40L levels (p=0.018)</td>
</tr>
<tr>
<td>Prospective, non-randomised study (185) Consecutive patients with stable CAD (n=103) Duration: 5 weeks</td>
<td>Stable CAD</td>
<td>ASA+Placebo vs ASA+Clopidogrel</td>
<td>sCD40L, RANTES, hsCRP</td>
<td>Plasma levels of sCD40L, RANTES, and hsCRP significantly reduced in ASA+Clopidogrel arm (p&lt;0.05, p&lt;0.01, and p&lt;0.01, respectively)</td>
</tr>
<tr>
<td>Prospective, randomised, double-blind study (107) Patients with stable CAD (n=73) Duration: 8 weeks</td>
<td>Stable CAD</td>
<td>ASA+Placebo vs ASA+Clopidogrel</td>
<td>sCD40L and hsCRP</td>
<td>sCD40L levels significantly reduced in ASA + clopidogrel arm (p=0.03) No difference regarding hsCRP levels</td>
</tr>
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</table>
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In healthy volunteers (105) and patients with PAD (104), clopidogrel reduced the formation of PLA through attenuation of CD62P expression. Clopidogrel also reduced agonist-induced monocyte CD11b expression on monocytes, which led to a reduction of leukocyte activation by adherent platelets (106). A subanalysis of the ASCET trial revealed, that patients with stable CAD presented with comparable reductions of the inflammatory markers CRP, TNF-α, IL-6, IL-10, MCP-1, CD40L, CD62P, and TGF-β at one year follow-up when they received either ASA or clopidogrel (97, 100). Of note, all patients included in the study were given ASA at

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<tr>
<td>Prospective, non-randomised, cross-sectional study (104) PAD patients (n=44) Healthy volunteers (n=9)</td>
<td>PAD</td>
<td>ASA vs Clopidogrel vs ASA+Clopidogrel</td>
<td>PLA, CD62P, MAC-1, I-CAM</td>
<td>Significant reduction of CD62P+, MAC-1 expression and PLA formation in clopidogrel and ASA + clopidogrel arm (p&lt;0.05) Soluble plasma ICAM-1 levels significantly reduced by ASA, clopidogrel, and ASA + clopidogrel (p&lt;0.05)</td>
</tr>
<tr>
<td>Prospective, randomised, double-blind study (142) Patients with stable CAD (n=110) Duration: 29 days</td>
<td>Stable CAD</td>
<td>ASA+Prasugrel vs ASA+Clopidogrel</td>
<td>CD62P, PLA</td>
<td>CD62P levels significantly lower in prasugrel treatment arm at 2 and 24 h, 14 and 29 days (p&lt;0.01) PLA formation significantly lower in prasugrel arm at 2 and 24 h, 14 and 29 days (p&lt;0.01)</td>
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<tr>
<td>Prospective study of the randomised, double-blind JUMBO trial (141) Patients undergoing PCI (n=131) Duration: 30 days</td>
<td>Patients undergoing PCI</td>
<td>ASA+Prasugrel vs ASA+Clopidogrel</td>
<td>CD62P, CD40L</td>
<td>CD62P and CD40L levels significantly lower in prasugrel arm at 24 h and 30 days (p&lt;0.05)</td>
</tr>
<tr>
<td>Prospective, double-blind, double-dummy, randomised trial (DISPERSE 2) (127) Patients with NSTE-ACS (n=990) Duration: 4 weeks</td>
<td>ACS</td>
<td>ASA+Clopidogrel vs ASA+Ticagrelor</td>
<td>IL-6, CRP, sCD40L, MPO</td>
<td>No significant difference in inflammatory biomarker measurements among treatment groups at baseline, discharge, and 4 weeks</td>
</tr>
<tr>
<td>Post-hoc analysis of the prospective, randomised PLATO trial (126) 18,421 PLATO patients with ACS treated with ticagrelor or clopidogrel Duration: 12 months</td>
<td>ACS</td>
<td>ASA+Clopidogrel vs ASA+Ticagrelor</td>
<td>CRP, IL-6</td>
<td>Fewer deaths attributed to sepsis in the ticagrelor arm (p=0.003) Lower leukocyte counts in clopidogrel arm during treatment (p&lt;0.0001 at 1, 3 and 6 months) but not at 1 month post-discontinuation CRP increased more at discharge in the ticagrelor arm (p&lt;0.001) Higher IL-6 during the first month of treatment with ticagrelor</td>
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<tr>
<td>PAR1 antagonists (Vorapaxar, Atopaxar) Prospective, non-randomised study (171) Patients with NSTE-ACS (n=85, n=41 in placebo arm, n=44 in vorapaxar arm)</td>
<td>ACS</td>
<td>Vorapaxar vs Placebo</td>
<td>CD62P, sCD40L, CRP, RANTES</td>
<td>Significant changes in the biomarker levels of CD62P, sCD40L, RANTES und CRP between the two treatment groups.</td>
</tr>
<tr>
<td>Prospective, randomised study (LANCELOT-CAD trial) (172) Patients with stable CAD (n=720 in either placebo or atopaxar arm) Duration: 24 weeks</td>
<td>Stable CAD</td>
<td>Atopaxar (50, 100, or 200 mg daily) vs Placebo</td>
<td>sCD40L, hsCRP, PIGF, Lp-PLA2, IL-6, IL-18</td>
<td>sCD40L decreased over time in the combined atopaxar group vs the placebo arm (p&lt;0.001) Increased Lp-PLA2 and IL-18 levels in the combined atopaxar group vs placebo (p&lt;0.001) No significant effect on the other inflammatory markers</td>
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In healthy volunteers (105) and patients with PAD (104), clopidogrel reduced the formation of PLA through attenuation of CD62P expression. Clopidogrel also reduced agonist-induced monocyte CD11b expression on monocytes, which led to a reduction of leukocyte activation by adherent platelets (106). A subanalysis of the ASCET trial revealed, that patients with stable CAD presented with comparable reductions of the inflammatory markers CRP, TNF-α, IL-6, IL-10, MCP-1, CD40L, CD62P, and TGF-β at one year follow-up when they received either ASA or clopidogrel (97, 100). Of note, all patients included in the study were given ASA at
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<tr>
<td>Prospective sub-study of the randomised, double-blind EPIC trial (161)</td>
<td>Patients undergoing PTCA (n=160)</td>
<td>Abciximab vs Placebo&lt;sup&gt;4&lt;/sup&gt;</td>
<td>CRP, IL-6, TNF-α</td>
<td>CRP and IL-6 levels increased significantly less at 24–48 h in abciximab arm (p=0.025 and p&lt;0.001, respectively) No significant differences between treatment arms at 4 weeks</td>
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<tr>
<td>Duration: 4 weeks</td>
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<tr>
<td>2 × 2 factorial randomised study (117)</td>
<td>Patients undergoing stenting were treated with either clopidogrel alone (300 mg or 600 mg; n = 60) or clopidogrel with eptifibatide (n = 60)</td>
<td>Clopidogrel vs Eptifibatide</td>
<td>CRP, TNF-α, CD62P, expression of activated GPIIb/IIIa</td>
<td>Significant reduction in platelet aggregation and active GPIIb/IIIa expression (p &lt; or = 0.001) in the clopidogrel + eptifibatide arm Decrease of CRP and TNF-α release (p &lt; or = 0.001) in the clopidogrel + eptifibatide arm</td>
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<tr>
<td>Duration: 24 h</td>
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<tr>
<td>Prospective, non-randomised study (186)</td>
<td>Consecutive PCI patients (n = 50)</td>
<td>Eptifibatide vs no Eptifibatide&lt;sup&gt;4&lt;/sup&gt;</td>
<td>CRP, IL-6</td>
<td>In eptifibatide treatment arm, CRP levels increased significantly less at 24 h (p&lt;0.05) No significant differences between arms regarding CRP at 48h or IL-6 at 24 and 48 h</td>
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<td>Duration: 48 h</td>
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<tr>
<td>Prospective, non-randomised study (187)</td>
<td>Patients undergoing PCI (n=45, abciximab or eptifibatide arm with n= 5), or no GP IIb/IIIa inhibitor; (n=15)</td>
<td>Abciximab (n = 15), Eptifibatide (n = 15), or no GP IIb/IIIa inhibitor (n = 15)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>RANTES, CD40L, sCD40L</td>
<td>Absence of GP IIb/IIIa inhibitor was associated with a small rise in sCD40L and RANTES post-PCI Eptifibatide significantly lowered baseline levels of sCD40L (p=0.018) and RANTES (p=0.008). This effect was not observed with abciximab.</td>
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<tr>
<td>Duration: 24 h</td>
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<tr>
<td>Placebo-controlled, randomised trial (161)</td>
<td>Patients undergoing PTCA (n=160)</td>
<td>Abciximab vs Placebo&lt;sup&gt;5&lt;/sup&gt;</td>
<td>CRP, TNF-α, IL-6</td>
<td>Less increase of CRP between baseline and 24 to 48 h in abciximab arm (p=0.025) Less increase of IL-6 and TNF-α in abciximab arm (p=0.001) At week 4, most marker levels had returned to baseline, with no significant differences between placebo and abciximab arms</td>
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<tr>
<td>Duration: 4 weeks</td>
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<tr>
<td>Prospective, randomised substudy of the GUSTO-IV trial (162)</td>
<td>Patients with NSTE-ACS, n=404</td>
<td>Abciximab vs Placebo&lt;sup&gt;6&lt;/sup&gt;</td>
<td>CRP, IL-6, Fibrinogen</td>
<td>CRP, IL-6, and fibrinogen increased their median levels (p&lt;0.001) in both arms There were no differences at any timepoint between the placebo and the abciximab arm.</td>
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<td>Duration: 72 h</td>
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1. All patients received ASA plus heparin or bivalirudin.  
2. All patients received ASA plus heparin.  
3. All patients received ASA plus low-molecular-weight heparin.  
4. There were 7 ASA + prasugrel recipients derived from the JUMBO trial and 124 ASA + clopidogrel recipients derived from a comparable historic cohort.  
5. All patients received ASA. Ticagrelor 90 mg or 180 mg twice daily, (with and without ticagrelor LD of 270mg) or clopidogrel 300 mg initially and then 75 mg/day.  
6. All patients received ASA plus heparin plus clopidogrel.  
7. All patients received ASA and clopidogrel.  
8. All patients received ASA and dalteparin.  
9. ACS – acute coronary syndrome; ADP – adenosine diphosphate; ALBION – Assessment of the best Loading dose of clopidogrel to Blunt platelet activation, Inflammation, and Ongoing Necrosis; ASA – acetylsalicylic acid; ASCET, ASpirin non-responsiveness and Clopidogrel Endpoint Trial; CAD – coronary artery disease; CD40L - CD40 ligand; CRP – C-reactive protein; EPIC – Evaluation of c7E3 for the Prevention of Ischemic Complications; ESPRIT – Enhanced Suppression of the Platelet IIb/IIIa Receptor with Integritin Therapy; GP – glycoprotein; GUSTO-IV – The Swedish Global Utilization of Strategies To open Occluded arteries-IV; h – hours; hsCRP – high-sensitivity CRP; ICAM-1 – intercellular cell adhesion molecule-1; IL-1β – interleukin-1β; IL-6 – interleukin-6; IL-10 – interleukin-10; IL-18 – interleukin-18; JUMBO – Joint Utilization of Medications to Block platelets Optimally; Lp-PLA2 – lipoprotein-associated phospholipase A2; MAC-1 – Macrophage-1 antigen, monocyte CD11b; MCP-1 – monocyte chemoattractantprotein-1; M-CSF – macrophage colony-stimulating factor; MI – myocardial infarction; NSTEMI – non-ST-elevation myocardial infarction; PAD – peripheral arterial disease; PAI-1 – plasminogen activator inhibitor-1; PCI – percutaneous coronary intervention; PLA – platelet-leukocyte aggregate; ; PlGF – placental growth factor; PTCA – percutaneous coronary angioplasty; RANTES – regulated upon activation, normal T-cell expressed, and secreted; s – soluble; TF-PCA – tissue factor procoagulant activity; TNF-α – tumour necrosis factor-α; TGF-β – transforming growth factor β; TRAP – thrombin receptor-activating peptide; vWF – von Willebrand factor; WARIS II – Warfarin Aspirin Reinfarction Study II. 

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the time of randomisation. Clopidogrel has also been shown to have a similar effect reducing serum levels of CD40L in stable CAD (107) and ACS (108, 109). In patients, who showed lower baseline levels of sCD40L and less platelet activation or patients with type 2 diabetes mellitus and no cardiovascular disease at study entry, clopidogrel treatment reduced plasma levels of RANTES leading to a suppression of monocyte activation, as RANTES mediates monocyte and lymphocyte recruitment to the sites of endothelial injury (110) (Table 3).

Several studies have demonstrated, that dual antiplatelet therapy (DAPT, clopidogrel plus ASA) reduces inflammatory markers to a greater extent than monotherapy (111, 112). In non-ST-segment elevation myocardial infarction (NSTEMI) patients’ high-sensitivity (hs)CRP and sCD40L levels were reduced only in those, who received the combination of clopidogrel and ASA (112).

Pretreatment with thienopyridines reduces death and MI after PCI (113). This is especially true in those patients with elevated baseline inflammatory markers like CRP (114). In the CURE trial, a 30% reduction of death, MI, or urgent target revascularisation was reported at 30 days in patients pretreated with clopidogrel and undergoing PCI (113). Clopidogrel pretreatment before PCI has demonstrated a 65% periprocedural reduction in the rise of hsCRP (115) and it has been associated with decreased agonist-induced platelet CD62P (116), sCD40L and a trend toward lower IL-6 levels 18–24 h after PCI compared to patients that were not pretreated. An increased inhibition of ADP-induced platelet aggregation and CD62P expression with high loading doses (LD) of clopidogrel was associated with decreased TNF-α and CRP levels compared to standard clopidogrel LD, suggesting a mechanistic link between the inhibition of platelet function, myonecrosis and inflammation (80, 117, 118). Recent evidence points to an inflammatory “rebound effect” after clopidogrel withdrawal in patients with diabetes and CAD. Angiolillo et al. (55) demonstrated a significant increase of ADP-induced platelet aggregation, of CRP- and CD62P-levels after clopidogrel withdrawal in 54 diabetic patients on long-term DAPT. This may partly explain why the use of ADP-receptor antagonists like clopidogrel is superior to ASA in secondary prevention of ischaemic events in diabetic patients (119); clopidogrel may hamper the effects of increased ADP exposure. Regardless of the clinical setting, the anti-inflammatory effect of clopidogrel is dependent on the baseline inflammatory state (120). The PROCLAIM study showed, that clopidogrel treatment with 300 mg LD followed by 75 mg daily maintenance dose plus ASA 81 mg daily significantly decreased sCD40L levels 24 hours (h) after stenting, whereas ASA treatment alone did not show an effect. The decrease in sCD40L sustained for 60 days, with rebound after drug discontinuation. The greatest decreases were seen in patients who had higher levels of inflammatory markers at enrollment (121).

Recent studies demonstrate that the P2Y12 receptor is expressed not only in platelets but also in other cell types, such as monocytes (122), VSMCs (123), dendritic cells (124), and lymphocytes (122). Therefore effects of ADP-antagonists are not only expected in platelets but also in the target cells mentioned above. Furthermore, other non-platelet dependent effects have been described for clopidogrel. Thus, Liverani et al. found that clopidogrel modulates lipopolysaccharide (LPS)-induced systemic inflammation through a P2Y12 receptor independent pathway (125).

### Ticagrelor

Ticagrelor is an orally available, directly acting, reversible inhibitor of P2Y12 receptors on platelets and provides greater inhibition of platelet aggregation than clopidogrel. The drug is metabolised by the CYP3A4 enzyme, and concomitant use with either strong CYP3A4 inhibitors or inducers is contraindicated. A subanalysis of PLATO showed a lower mortality following pulmonary adverse events and sepsis within the ticagrelor arm compared to clopidogrel (126). There are only limited data available on ticagrelor’s influence on inflammatory markers in vivo. Husted et al. could demonstrate that a treatment with ticagrelor and clopidogrel did not lead to significant differences regarding the inflammatory biomarkers CRP, IL-6, MPO, and sCD40L in patients with NSTE-ACS among treatment groups at baseline, discharge, and at four weeks (127) (Table 3). Another effect of ticagrelor is, that it increases adenosine plasma concentration in patients with ACS (128). This effect is mediated by blocking cellular uptake of adenosine through inhibition of equilibrative nucleoside transporter 1 (ENT1) (129). Adenosine is one of the key molecules that regulate numerous physiological processes by activating four G-protein-coupled adenosine receptors (ARs), A1, A2A, A2B, and A3 ARs. Adenosine acts with a concentration dependent affinity on these receptors. For example, at low concentrations, adenosine predominantly acts on high affinity leukocyte A1 receptors thereby exerting pro-inflammatory properties. Downregulation of pro-inflammatory mediators is mainly mediated via A2A and A2B AR (130–132). Adenosine is a potent endogenous anti-inflammatory and immunoregulatory molecule. In the setting of inflammation, the generation and release of adenosine is greatly enhanced. Neutrophils that play an important role in host defense, especially in acute phase response, also release adenosine. On the other hand, adenosine itself regulates neutrophil chemotaxis and phagocytosis and serves as a modulator of neutrophil functions, such as granule release and oxidative burst in a concentration dependent manner playing a pivotal role, for instance, in ischaemia reperfusion injury and sepsis (128). When adenosine is physiologically released from cells at sites of inflammation or tissue injury, it regulates the immune and inflammatory systems and plays a central role in wound healing by increasing angiogenesis through upregulation of vascular endothelial growth factor (VEGF) (131). Despite these beneficial properties, adenosine has an extremely short half-life because of its rapid metabolism in blood due to conversion by adenosine kinase to adenosine monophosphate (AMP) or its change to inosine by adenosine deaminase. This pharmacological instability prevents its clinical usage for longterm treatment (130–132). Furthermore, Rahman et al. could show that Ticagrelor reduces neutrophil recruitment and lung-damage in an abdominal sepsis model. Pulmonary infiltration of neutrophils at 24 h was reduced by 50% in ticagrelor-treated mice. Moreover, ticagrelor abolished CLP-provoked lung oedema and decreased lung damage score by...
41%. Notably, ticagrelor completely inhibited formation of platelet-neutrophil aggregates and markedly reduced thrombocytopenia in CLP animals. In addition, ticagrelor reduced platelet shedding of CD40L in septic mice (133). As for prasugrel and clopidogrel, the anti-inflammatory effects of ticagrelor can be explained by indirect effects by effective platelet inhibition. Hence, additionally to adenosine-mediated effects, there might also be direct anti-inflammatory effects of ticagrelor, which are of clinical relevance and subject of further investigation.

A recent study showed in a rat model, that constrictions of rat tail arteries induced with a stable analogue of ADP, 2-MeS-ADP, was inhibited, when the animals were pre-treated with ticagrelor. This effect was not observed for clopidogrel or prasugrel. These observations suggest that ticagrelor prevents ADP-induced contraction of VSMCs (134). The same group showed, that pretreatment with high- but not low-dose ASA enhanced the reactivity of VSMCs only in vessels containing endothelial cells in a rat model. The suppression of 2-MeS-ADP-induced VSMC contraction by ticagrelor observed in arteres with and without endothelium could only be found in arteries with endothelium pretreated with low-dose ASA. For endothelium-denuded vessels a significant reduction of the maximal effect of ticagrelor was observed (135). Thus, a dose-dependent influence of ASA on ticagrelor’s effect on the endothelium can be suggested.

Another study evaluated pro- and anti-inflammatory effects of platelet inhibition by ticagrelor, which have been reported in animal models. In a set of in vitro experiments, peripheral blood mononuclear cells (PBMC) incubated with the TLR2 ligand Pam3CSK4 produced less cytokines in the presence of platelets, whereas platelets increased the production of cytokines, when PBMCs were exposed to TLR4 ligand LPS. These effects of platelets were dependent on direct platelet-leukocyte aggregation and for the Pam3CSK4-induced response, on phagocytosis of platelets by monocytes. In a double blind, placebo-controlled crossover trial in healthy volunteers, a single oral dosage of 180 mg ticagrelor reduced platelet-monocyte complex formation. This was associated with an increase in pro-inflammatory cytokines in blood exposed to Pam3CSK4, but a decrease in these cytokines in blood exposed to LPS. These findings show that platelets differentially modulate TLR2- and TLR4-mediated cytokine responses of PBMCs. Through inhibition of platelet-leukocyte interaction, P2Y12 receptor antagonists may either exert a pro- or anti-inflammatory effect during infections depending on the TLR primarily involved (136).

Prasugrel
Prasugrel is a third generation thienopyridine targeting the P2Y12-ADP receptor on platelets in the same way as clopidogrel does. The prodrug prasugrel inhibits platelets irreversibly. Compared to clopidogrel, prasugrel provides a faster onset of action and less variability of platelet responsiveness due to fewer metabolisation steps leading to higher plasma levels and faster availability of the active metabolite. Frelinger et al. analysed anti-inflammatory mechanisms in a sub-analysis (137) and showed that in vitro the active metabolite inhibits ADP-stimulated inflammatory markers of platelet activation along with blocking of platelet-platelet aggregation, monocyte-platelet aggregation, neutrophil-platelet aggregation, reduced platelet surface CD62P, and activated GPIIb/IIIa (137, 138).


Other in vitro data in mice, in the blood from healthy volunteers (139, 140) and in few clinical studies provide limited data on further anti-inflammatory effects of prasugrel. In a subanalysis of the JUMBO trial, ACS patients undergoing PCI received prasugrel and ASA. The levels of CD62P and CD40L at 24 h and 30 days were significantly lower than at baseline in this subgroup, and also lower compared to a group of patients treated with clopidogrel (141). Another randomised study of 110 ASA recipients with stable CAD showed, that the prasugrel arm presented with significantly decreased platelet-leucocyte conjugates formation and significantly lower levels of CD62P over time compared to the clopidogrel arm (142). The pronounced anti-inflammatory effect of prasugrel on the analysed cytokines is probably caused by a significantly greater degree of platelet inhibition with less inter- and intraindividual variability compared to clopidogrel, and may contribute to its significant benefit in reducing the risk of recurrent thrombotic events in patients with ACS who undergo PCI (143). Interestingly, the active metabolite of prasugrel has been shown to inhibit platelet-leucocyte interaction as well as procoagulant activity in in vitro studies in mice and analyses conducted in blood from healthy volunteers (137, 140). It was also shown, that prasugrel led to a decrease of MAC-1 and CD62P (138, 139) (Table 3). Furthermore, prasugrel also mediates non-platelet dependent effects. It has been shown that prasugrel metabolites inhibit neutrophil functions through neither the P2Y12 nor P2Y13 receptor in neutrophils (144).

Rebound phenomenon after cessation of antiplatelet therapy
The rebound effect after cessation of antiplatelet therapy with clopidogrel remains controversial. Although clinical guidelines for the treatment of CAD and ACS recommend ASA lifelong and clopidogrel for a period up to 12 months, depending on the indication, the optimal duration of clopidogrel therapy actually remains contentious. Premature cessation of clopidogrel after drug-eluting stent (DES) implantation is an established risk factor for stent thrombosis, but recent clinical studies have also demonstrated a link between “on time” clopidogrel withdrawal and clustering of ischemic events within 90 days after drug termination.
This phenomenon is called „rebound“ prothrombotic and/or proinflammatory response. One study showed that, there is a transient rebound leading to increase of platelet reactivity within three months. Patients received ASA and clopidogrel for at least one year after DES. The discontinuation of clopidogrel increased platelet aggregation and in fact was significantly more increased at one month compared to the three months measurements. This suggests that, there is a transient increase in platelet reactivity one month after clopidogrel withdrawal. This phenomenon may partly explain described clustering of thrombotic events after clopidogrel discontinuation (145).

Another retrospective cohort study of 3,137 patients with ACS and post-discharge clopidogrel treatment reported a clustering of adverse events in the initial 90 days after clopidogrel discontinuation among both medically treated and PCI-treated patients, supporting the possibility of a clopidogrel rebound effect (146).

A „rebound“ phenomenon associated with clopidogrel cessation is commonly defined as

- Occurrence of an adverse clinical event shortly after, and because of clopidogrel discontinuation,
- Another aspect of the termination of long-term clopidogrel therapy is the proinflammatory response. Increased levels of platelet reactivity and biomarkers including ADP-induced PA, hsCRP and CD62P were reported by Angiolillo et al. after cessation of clopidogrel in a cohort of diabetic patients (55). The DECADES study (147) examined the effect of clopidogrel cessation on inflammation 12 months after DES-implantation in a non-diabetic population. A significant increase of sCD40L and CD62P was observed between two and four weeks after clopidogrel withdrawal. In another study on 200 patients undergoing PCI, the hsCRP, sCD40L and lipid levels (triglycerides, cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL)) were evaluated on the day of clopidogrel cessation and 45 days after treatment. In men (n=151), the sCD40L serum levels were significantly higher 45 days after clopidogrel discontinuation (p=0.007), while the hsCRP levels were not significantly different (p=0.407). Therefore, clopidogrel withdrawal was associated with a pronounced proinflammatory response, and also with a lower HDL concentration in these patients (148).

- Increased platelet activation resulting in a pro-thrombotic tendency due to the direct loss of the clopidogrel effect on the inhibition of ADP-induced platelet aggregation as well as a loss of synergistic effects of clopidogrel on the inhibition of AA-induced platelet aggregation, which is predominantly affected by ASA, might also play an important role (149). Recent studies showed an additional antiplatelet activity via the AA-TRX2-COX pathway potentiating the effects of ASA, which implies that cessation of clopidogrel affects both, the ADP- and AA-induced platelet aggregation (55, 150–153) contributing to the rebound effect.

In contrary, a randomised study called Abrupt versus Tapered Interruption of Chronic Clopidogrel Therapy after Drug-Eluting Stent Implantation (ISAR-CAUTION), found no evidence of rebound effects after tapering or abrupt termination of clopidogrel in patients with stable CAD. In this study, 69 patients receiving clopidogrel prior to DES implantation were randomised to a tapering regimen for four weeks or to abrupt discontinuation of clopidogrel. Multiple measurements of platelet reactivity/aggregation showed, that levels returned to baseline shortly after cessation in both arms. Therefore, this study confirms, that tapering of antiplatelet therapy with clopidogrel is not necessary as discussed before, as it does not result in values of platelet aggregation that are lower than those seen when the antiplatelet medication is stopped abruptly (154, 155).

Just recently, the debate about a possible rebound effect has been heated up by an observed clustering of MI within 90 days of prasugrel discontinuation in both treatment groups (12 vs 30 months prasugrel duration) in the Taxus Liberte-Post Approval substudy of the DAPT trial (156).

### GPIIb/IIIa Inhibitors

GPIIb/IIIa Inhibitors reduce platelet aggregation and thrombus formation by binding to the activated GPIIb/IIIa platelet receptor. Abciximab, eptifibatide, and tirofiban markedly decrease the risk of ischemic complications post-PCI (157–159). There are structural and functional differences also including the binding sites among GPIIb/IIIa inhibitors, which reflect the differences in their efficacy [89]. Furthermore, abciximab has higher affinity for the GPIIb/IIIa receptor and binds to other receptors, such as vitronectin and MAC-1 (CD11b) receptors. There is also clinical evidence that especially high risk CAD and ACS patients benefit more from GPIIb/IIIa inhibitors than low or medium risk stable CAD patients (160). Several clinical studies suggest that blockade of the GPIIb/IIIa receptor also limits the inflammatory response secondary to PCI, but the results could not be proven consistently in repeated studies.

A sub-analysis of the EPIC trial demonstrated that abciximab significantly suppressed the periprocedural rise of hsCRP, IL-6, and TNF-α observed in patients undergoing PCI. 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 (161). But the results of the EPIC trial could not be confirmed in the GUSTO IV-ACS trial which showed that the 24-48 h infusion of abciximab had no effect on the inflammatory markers IL-6, CRP, and fibrinogen at all (162) (Table 3). Eptifibatide infusion in PCI patients was reported to reduce levels of sCD40L and RANTES after PCI (163). In patients with NSTE-MI, a 48-h infusion of tirofiban along with a combination treatment of ASA, clopidogrel, a statin, and unfractionated heparin attenuated the rise in hsCRP at 48 h and 72 h compared with standard therapy alone (164) (Table 3). On the other hand, tirofiban administration in patients with stable CAD undergoing PCI did not show any significant effect on various inflammatory markers (165). These results suggest that the benefit is related to baseline levels of inflammation depending on the clinical scenario.
Cangrelor

Cangrelor is a direct-acting, intravenous, reversible, competitive inhibitor of the P2Y12 receptor. It has a rapid onset and short half-life of approximately 3 minutes and is metabolised in the plasma. It does not require hepatic conversion, has a dose-dependent pharmacokinetic profile, and reaches steady state within minutes (166, 167). The clinical effects of cangrelor in comparison with placebo or clopidogrel respectively in CAD patients were investigated in three major randomised, controlled trials, CHAMPION-PCI, PLATFORM, and PHOENIX trial. The compound was also tested in a pharmacodynamic study (BRIDGE trial) for bridging antiplatelet therapy in patients undergoing coronary artery bypass surgery.

There is only limited data available on anti-inflammatory effects of cangrelor in combination with ASA and abciximab or tirofiban (168). Cangrelor inhibited TRAP-induced responses. Furthermore, a decrease of sCD40L was reported in healthy volunteers (▶Table 3).

PAR-1 Antagonists

Vorapaxar (SCH 530348)

Vorapaxar is a PAR-1 inhibitor and was investigated for the treatment and prevention of atherothrombotic events in patients with ACS, cerebrovascular disease, and PAD. The agent is metabolised in the liver through CYP enzyme pathways and has an estimated half-life of 126-269 h. The compound was tested in combination with ASA and/or clopidogrel in RCTs. The TRACER trial was a phase III, prospective, randomised, double-blind, placebo controlled secondary prevention trial evaluating the early administration of a LD of vorapaxar 40 mg, followed by 2.5 mg/day compared with placebo for one year in approximately 13,000 patients with recent NSTEMI (169). The TRA2P–TIMI 50 trial was a phase III, randomised, double-blind, placebo-controlled study enrolling 26,447 patients with a previous stroke, MI, or PAD assessing vorapaxar 2.5 mg/day compared with placebo, in addition to ASA and/or clopidogrel for one year for secondary prevention (170).

In a substudy of TRACER 249 patients were enrolled. Platelet aggregation was assessed in 85 subjects (41 placebo, 44 vorapaxar) along with platelet PAR-1 expression and inflammatory biomarkers before and during treatment. LTA responses to TRAP and a combination of collagen-related peptide + ADP + TRAP as well as VerifyNow platelet aggregation units (PAU) were markedly inhibited by vorapaxar. In contrast to the placebo group, PAR-1 receptor number in the vorapaxar group at one month was significantly lower than at baseline (179 vs 225; p=0.004). There were significant changes in selected inflammatory biomarker levels like RANTES between the two treatment groups (171) (▶Table 3).

Biomarker release under PAR-1 antagonism using vorapaxar in combination with DAPT was recently investigated in a small substudy of the TRACER study in ACS patients. There was a marked reduction of some inflammatory biomarkers (MPO, RANTES, microparticles) under short and long-term treatment, however this reduction was also observed in the placebo arm. Therefore, the anti-inflammatory effects cannot be directly attributed to specific properties of vorapaxar (171).

Atopaxar

Atopaxar, another PAR-1 antagonist, that entered the clinical trial program, but whose development has been discontinued, decreased sCD40L levels but not other inflammatory markers including hsCRP, myeloperoxidase placental growth factor (PIGF) and IL-6. Atopaxar was also associated with higher lipoprotein-associated phospholipase A2 (Lp-PLA2) and IL-18 levels compared to placebo (172).

Indirect versus direct antinflammatory effects of antiplatelet therapy

To explain the anti-inflammatory impact mediated by antiplatelet agents, direct and indirect effects have been hypothesised. The indirect hypothesis suggests, that the effects on inflammatory markers are secondary to decreased platelet activation due to effective platelet inhibition. There are several data supporting the findings in which inflammatory markers are reduced secondary to platelet activation and inhibition. This hypothesis is supported by studies that highlight a more pronounced effect of antiplatelet therapies on inflammatory markers in patients with ACS or patients with a high risk for recurrent events compared to healthy controls. For example, in a high-risk patient collective with type 2 diabetes mellitus, clopidogrel withdrawal after longer-term DAPT was associated with an increase in both platelet activation and inflammatory biomarkers (55).

The direct hypothesis suggests, that antiplatelet agents exert their effects directly on particular inflammatory pathways. There are few data to support the direct hypothesis by showing a decrease of distinct inflammatory markers in stable CAD or healthy individuals independently from platelet inhibition (97, 173, 174). In vitro ASA (81) and clopidogrel (175) directly block NF-kB in endothelial cells. This transcription factor can regulate the expression of genes involved in the immune response. GPIIb/IIa inhibitors can cause the same effect in stimulated neutrophils plated on fibronectin (176). Levels of the inflammatory markers CD40L, endothelial nitric oxide synthase, and TF were reduced almost completely to control levels within 24 h of clopidogrel administration, while platelet inhibition was only partially reduced in this timeframe in a rabbit model of MI (177). Currently available data suggest, that most of the anti-inflammatory effect of antiplatelet agents is mediated indirectly through the inhibition of platelet activation. Evidence supporting this hypothesis comes from the CHARISMA trial (112, 178) including patients with stable CAD, who have already been treated with ASA. An additional treatment with clopidogrel did not provide significant clinical benefit for the overall population. Post-hoc analyses revealed that clopidogrel was clinically beneficial in patients with a history of MI, stroke, or symptomatic PAD. These patients at risk exhibit a higher degree of

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platelet activation and enhanced inflammatory state, which could be indirectly prevented by the additional antiplatelet effect of clopidogrel. Therefore, effective platelet inhibition plays a pivotal role in stable CAD and ACS to avoid recurrent ischaemic events by a concomitant reduction of platelet activation and inflammation to prevent progression of atherosclerosis. Testing of platelet function and inflammatory markers over time might help to identify patients at risk, who need a more pronounced platelet inhibition after PCI or ACS. Especially effective combination therapy of antiplatelet drugs and drug with anti-inflammatory capacities represents a promising, thus an individualised risk adjusted therapy. In this context, statins by exerting anti-inflammatory effects attenuate risk for recurrent ischaemic events in cardiovascular patients (179) and might have a vice versa inhibitory effect on platelet function mediated by anti-inflammatory properties (180).

Conclusion and future perspectives

Vascular inflammation is the central underlying mechanism in atherogenesis and atheroprogression. Platelets are key mediators in the initiation and maintenance of a chronic proinflammatory and prothrombotic milieu by direct interactions with inflammatory cells and secretion of autocrine and paracrine effector molecules. Beyond this, recent experimental studies document that platelets are critically involved in autoimmune diseases and innate immunity (188-190). Data from the animal and human model systems suggest that modification of platelet function may also modulate expression of established inflammatory mediators like CD40L, CD62P, 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 (the number of patients enrolled, whether they had stable CAD or ACS, and how markers were measured using different assays and materials), the accumulated data support the hypothesis, that antiplatelet therapies may reduce vascular inflammation in patients with atherosclerosis. Therefore, antiplatelet therapy may help to break the cycle of cardiovascular disease - not only by inhibiting platelet activation and aggregation, but also by limiting local and systemic inflammatory responses. Results of clinical studies have shown, that different antiplatelet agents may affect different inflammatory markers. To explain the anti-inflammatory effects mediated by antiplatelet agents, direct and indirect effects have been hypothesised. Overall, it is likely that most of the anti-inflammatory properties of antiplatelet agents are a result of decreased platelet activation. The question of, whether the anti-inflammatory effect of antiplatelet agents is mediated mainly by an indirect or direct effect, cannot be fully answered from currently available data. Therefore, future systematic studies are needed with both established and novel antiplatelet agents, to further understand the relationships between antiplatelet therapy and vascular inflammation and to improve the clinical outcome of patients at risk for recurrent cardiovascular events. Novel techniques like proteomics of platelet releasates (181) and platelet RNA-profiling (182) could help to better understand specific anti-inflammatory effects of antiplatelet therapies.

Furthermore, testing of platelet function and inflammatory markers over time might help to identify patients at risk, who need a more pronounced and prolonged platelet inhibition beyond current guidelines as demonstrated by recent RCTs (191, 192). An effective combination therapy of anti-platelet drugs and drugs with anti-inflammatory capacities might represent a promising approach and should be investigated for individualised therapeutic concepts in the future.

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