Article published online: 2022-07-29

Platelets in Myocardial Ischemia/Reperfusion Injury

Nancy Schanze[1,2] Muataz Ali Hamad[1,3,4] Thomas Georg Nührenberg[5,6] Christoph Bode[1] Daniel Duerschmied[1,2,7]

[1] Department of Cardiology and Angiology I, Heart Center, University of Freiburg, Freiburg, Germany
[2] Department of Cardiology, Angiology, Haemostaseology and Medical Intensive Care, University Medical Centre Mannheim, Medical Faculty Mannheim, Heidelberg University, Germany
[3] Spemann Graduate School of Biology and Medicine (SGBM), University of Freiburg, Freiburg, Germany
[4] Faculty of Biology, University of Freiburg, Freiburg, Germany
[5] Department of Cardiology and Angiology II, Heart Center, University of Freiburg, Freiburg, Germany
[6] Institute for Experimental and Clinical Pharmacology and Toxicology, University of Freiburg, Freiburg, Germany
[7] European Center for AngioScience (ECAS) and German Center for Cardiovascular Research (DZHK) partner site Heidelberg/Mannheim, Mannheim, Germany

Address for correspondence Daniel Duerschmied, Univ.-Prof. Dr. med., Department of Cardiology, Angiology, Haemostaseology and Medical Intensive Care, University Medical Centre Mannheim, Medical Faculty Mannheim, Heidelberg University, Germany Theodor-Kutzer-Ufer 1-3 68167 Mannheim (e-mail: daniel.duerschmied@umm.de).

Hamostaseologie 2023;43:110–121.

Abstract

Coronary artery disease, including myocardial infarction (MI), remains a leading cause of global mortality. Rapid reperfusion therapy is key to the improvement of patient outcome but contributes substantially to the final cardiac damage. This phenomenon is called "ischemia/reperfusion injury (IRI)." The underlying mechanisms of IRI are complex and not fully understood. Contributing cellular and molecular mechanisms involve the formation of microthrombi, alterations in ion concentrations, pH shifts, dysregulation of osmolality, and, importantly, inflammation. Beyond their known action as drivers of the development of coronary plaques leading to MI, platelets have been identified as important mediators in myocardial IRI. Circulating platelets are activated by the IRI-provoked damages in the vascular endothelium. This leads to platelet adherence to the reperfused endothelium, aggregation, and the formation of microthrombi. Furthermore, activated platelets release vasoconstrictive substances, act via surface molecules, and enhance leukocyte infiltration into post-IR tissue, that is, via platelet–leukocyte complexes. A better understanding of platelet contributions to myocardial IRI, including their interaction with other lesion-associated cells, is necessary to develop effective treatment strategies to prevent IRI and further improve the condition of the reperfused myocardium. In this review, we briefly summarize platelet properties that modulate IRI. We also describe the beneficial impacts of antiplatelet agents as well as their mechanisms of action in IRI beyond classic effects.

Keywords

- myocardial infarction
- ischemia reperfusion injury
- platelets
- reperfusion
- ischemia

received
June 15, 2021
accepted after revision
January 13, 2022

DOI https://doi.org/10.1055/a-1739-9351.
ISSN 0720-9355.

Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

Introduction

Platelets are anucleate cells that play a primary role in hemostasis. On top of that, platelets have been recognized as potent immunomodulators that can secrete numerous factors and express a variety of surface molecules to modulate leukocyte functions (e.g., phagocytosis or extravasation).[1] For example, platelets secrete chemokines like RANTES/C-C motif ligand (CCL)5, serotonin, or platelet factor 4 (PF4)/C-X-C motif ligand (CXL)4 at sites of inflammation to recruit leukocytes.[2–6] To establish direct cell–cell interactions with leukocytes or endothelial cells, platelets can secrete or expose adhesion proteins such as P-selectin, fibronectin, fibrinogen, or von Willebrand factor (VWF).[7–14] Furthermore, platelets can activate the complement system and are even able to directly capture and neutralize pathogens.[1,15]

Coronary artery disease (CAD), including myocardial infarction (MI), together with stroke, remains the leading cause of global mortality.[16] Rapid reperfusion via percutaneous coronary intervention (PCI) significantly improves the prognosis of patients suffering from acute MI. However, this sudden reperfusion causes adverse effects itself and substantially contributes to the myocardial damage.[17,18] This is referred to as ischemia/reperfusion injury (IRI) and was reported to account for nearly 50% of the final infarct size in acute MI.[19] IRI is associated with no-reflow, myocardial stunning, and arrhythmias.[18,20] The cellular and molecular mechanisms underlying IRI are complex and include inflammation, endothelial dysfunction, alterations in calcium concentrations, and pH, as well as mitochondrial dysfunction and oxidative stress.[18,21] Furthermore, unregulated alterations in cytosolic osmolality and cell volume provoke cellular and interstitial edema leading to microvascular obstruction.[22,23]

IRI is accompanied by leukocyte infiltration and inflammation.[24,25] An overshoot of this inflammatory reaction (which is actually necessary for the cardiac tissue repair) can worsen the initial injury.[25,26]

Platelets are known to play a key role in the growth of coronary plaques and the thrombotic occlusion of coronary vessels that cause ischemia and MI.[27] But platelets are also central players in the course of myocardial IRI (►Fig. 1). Circulating platelets are activated by the IRI-provoked damages in the vascular endothelium.[28] Subsequently, activated platelets adhere to the reperfused endothelium, aggregate, and form microthrombi.[29] They also release vasoconstrictive substances[29] and activated platelets in platelet–leukocyte complexes (PLCs) enhance leukocyte infiltration into the post-IR tissue.[30] In addition, it is likely that ischemia and reperfusion themselves influence certain platelet properties relevant to IRI by altering oxygen concentration.[31,32]

In this review, we briefly summarize platelet properties that modulate IRI. Additionally, we describe the beneficial

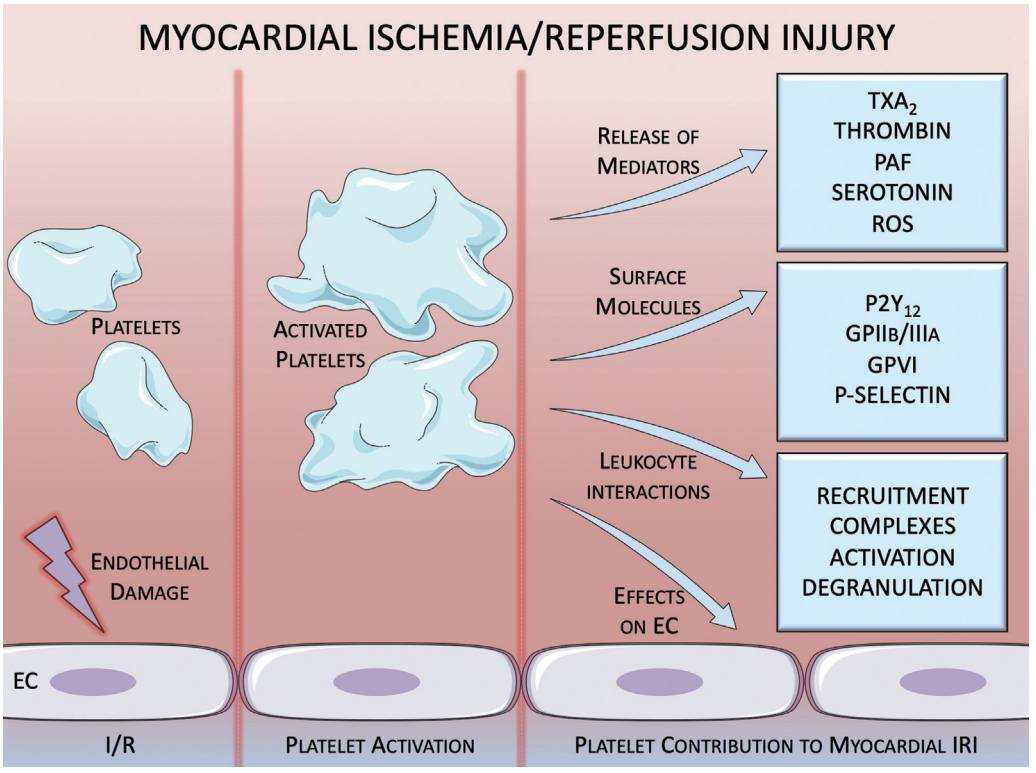

Fig. 1 Platelets in myocardial ischemia/reperfusion injury (IRI). IRI-provoked endothelial damage causes platelet activation. During platelet activation, adhesion proteins, e.g., P-selectin, are expressed or exposed and facilitate interaction with leukocytes, leading to the formation of platelet–leukocyte complexes. This leads to recruitment of leukocytes to the site of inflammation as well as their activation and degranulation. Other platelet surface molecules that are important mediators in IRI are glycoprotein (GP) IIb/IIIa, GPVI, and P2Y12. Furthermore, secretable factors, e.g., serotonin, thrombin, and platelet activating factor (PAF) in high concentrations, aggravate cardiac damage. TXA2, thromboxane A2; EC, endothelial cell; I/R, ischemia/reperfusion; IRI, ischemia/reperfusion injury. The Figure was created using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

impacts of antiplatelet agents as well as their mechanisms of action in IRI beyond classic effects.

Thromboxane A_2

Cyclooxygenases (COX) catalyze the conversion of arachidonic acid to prostaglandin G_2 and H_2. This is the prerequisite for subsequent generation of thromboxane A_2 (TXA_2) via thromboxane synthase.[33] Binding of TXA_2 to its receptor on platelets leads to platelet-shape change, inside-out activation of integrins, and degranulation, as well as intracellular Ca^{2+} increase.[33] TXA_2 is considered a pro-aggregatory prostanoid and therefore is implicated in platelet-dependent thrombotic (re-)occlusion of the culprit atherosclerotic lesion, stent thrombosis, and reduced microcirculatory flow.[34] Furthermore, TXA_2 mediates effects through its receptor on other cell types, that is, within the vasculature where it contributes to vasoconstriction.[35] Aspirin, the first antiplatelet drug to be clinically used, irreversibly inhibits primarily COX1 (which is constitutively expressed in platelets) by acetylation and thereby interrupts the synthesis pathway generating TXA_2.[34] Already decades ago, the "Second International Study of Infarct Survival" (ISIS-2) found convincing evidence for the efficacy of aspirin monotherapy in the treatment of patients at the onset of acute MI.[36] And to date, aspirin in combination with $P2Y_{12}$ inhibitors is recommended for acute coronary syndrome treatment.[37] Beyond its inhibitory effect on COX enzymes, aspirin was reported to reduce PF-3 and -4 as well as coagulation factors II, VII, IX, and X (at 200 mg) and to have fibrinolytic activity at very high doses (1,800 mg).[38,39] Direct evidence for an interference of aspirin with molecular mechanisms specifically underlying myocardial reperfusion injury are rare, but secondary effects of platelet inhibition including reduced reactive oxygen species (ROS) and inflammatory cytokines as well as improved endothelial function seem to contribute to the beneficial effect.[35] Furthermore, a recent study on rats showed that in the setting of transient ligation of the left anterior descending coronary artery (LAD), aspirin treatment 10 minutes before reperfusion results in cardioprotection (i.e., less apoptosis, improved function, and decreased infarct size) through activation of JAK2/STAT3 signaling in myocardial tissue.[40]

Thrombin

Thrombin, the key effector of the coagulation cascade, acts on platelets by cleaving protease activated receptor (PAR)1 and PAR4 (respectively PAR3 and PAR4 in mice).[41] This leads to platelet degranulation and the accompanying release of molecules including thrombin itself and adenosine diphosphate (ADP) as well as serotonin and P-selectin (the role of the latter two in IRI will be explored in other articles of this review). Thrombin is also a potent activator of platelet integrin glycoprotein (GP)IIb/IIIa and thereby drives rapid platelet aggregation.[41] Other than directly acting on platelets, thrombin cleaves fibrinogen into fibrin as well as multiple PARs on endothelial cells and leukocytes.[41] The activation of PARs leads to upregulation of endothelial adhesion molecules and proinflammatory cytokines.[42] Altogether, thrombin is a major player in hemostasis and thrombosis as well as a modulator of the inflammatory response. Microvascular thrombosis with associated inflammation is well recognized in the context of IRI.

Bivalirudin is a direct and specific thrombin inhibitor which is successfully used for anticoagulation in patients undergoing PCI for MI.[43] In vitro data suggest that on top of mere anticoagulation, bivalirudin leads to reduced thrombin-dependent platelet PAR activation[44] and may exert an anti-inflammatory effect via a reduction of soluble CD40 ligand (sCD40L) during PCI.[45] In a mouse study, the inhibition of thrombin generation through the TF pathway ameliorated I/R by decreasing chemokine expression and leukocyte infiltration.[46]

Another opportunity to suppress the action of thrombin is the blockade of its receptors. An advantage of blocking PARs instead of thrombin itself is that thrombin-dependent fibrin generation is preserved. This may reduce bleeding complications. Preclinical studies show effectiveness of PAR inhibition in experimental MI models. On the one hand, PAR4 deficiency was demonstrated to result in cardioprotection in the early phase after acute MI in mice in part by reducing early inflammatory signals and myocyte apoptosis.[47,48] On the other hand, PAR4 deficiency was demonstrated to impair myocardial healing after chronic MI leading to increased cardiac rupture and mortality. Mechanistically, this was explained by altered neutrophil properties that prevent normal reparative processes to resolve the inflammatory response.[48] Such data suggest that permanent PAR4 inhibition should be viewed with caution. Clinical trials demonstrated that PAR1 inhibition with vorapaxar (which leads to interruption of thrombin-mediated platelet aggregation) in patients with previous MI is effective in the secondary prevention of recurrent thrombotic events, albeit at the expense of an increase in major bleedings.[49]

Platelet activity is strongly interwoven with coagulation. For example, activated platelets secrete coagulation factor V which in its activated form interacts with activated factor X.[50] In the common pathway of coagulation, factor X is directly linked to thrombin generation. Additionally, its active form, factor Xa, has independent effects through PAR activation.[51] Anticoagulation with the factor Xa inhibitor rivaroxaban in patients with recent acute coronary syndrome reduces the risk of death from cardiovascular causes, MI, or stroke.[52,53] Beyond its main effect as an anticoagulant, rivaroxaban also has pleiotropic effects including inhibition of PAR-mediated platelet activation, inhibition of PAR-mediated inflammation, and PAR-mediated fibroblast activity.[52] Evidence from a preclinical study suggests that the secondary prevention of cardiovascular events after myocardial IRI in mice was in part mediated by reduced inflammation and fibrosis in the left ventricle.[54]

Factor XI (FXI) belongs to the intrinsic pathway of the coagulation cascade and is believed to play an important role in thrombosis but only a minor role in hemostasis. Of note, thrombin can enhance its own generation through a positive feedback loop involving FXI.[41] It was shown that the intrinsic coagulation pathway contributes to myocardial IRI. In a

model of transient LAD ligation in mice, myocardial IRI was partially attenuated by FXI inhibition. The FXI inhibitor that was applied in this study (14E11) has anti-inflammatory properties in addition to its antithrombotic properties. Therefore, cardioprotection was likely mediated by reducing contact activation, thrombin generation, inflammation, or a combination of these mechanisms.[55] FXI inhibition may provide a novel mechanism for anticoagulation, without increasing the risk of clinically significant bleeding. Phase II clinical trials for FXI inhibition (on top of a dual-antiplatelet therapy) in patients following acute MI are ongoing.[56]

$P2Y_{12}$ Receptor

Activation of the platelet $P2Y_{12}$ receptor by ADP is important in platelet aggregation and amplifies the platelet response to other agonists. Consequently, inhibition of $P2Y_{12}$ has an antiaggregatory effect and is beneficial in the treatment of MI. Several $P2Y_{12}$ inhibitors are available, such as the thienopyridines clopidogrel and prasugrel which have to be metabolically activated and the adenosine triphosphate analogs cangrelor and ticagrelor.

In vivo and ex vivo studies applying $P2Y_{12}$ inhibitors in dogs,[57] rats,[58,59] or rabbits[60] show improved cardiac outcome after experimental MI in terms of infarct size, tissue perfusion, and cardiac function. Clinical trials also clearly show improved outcome in MI patients undergoing $P2Y_{12}$ inhibition.[61–64] Consequently, $P2Y_{12}$ antagonists in combination with aspirin are standard of care in patients undergoing PCI for the management of acute MI in clinical practice.[37]

In addition to mere antiaggregatory effects of the $P2Y_{12}$ inhibition, specific pharmacological (off-target) effects of $P2Y_{12}$ inhibitors were described. These properties provide a potential additional positive effect on the reperfused myocardium and are therefore of interest for clinical practice.[65]

It was shown that cangrelor and clopidogrel treatment, in addition to inhibiting ADP-dependent platelet aggregation and recruitment, decreased platelet P-selectin expression and platelet–leukocyte interactions in CAD patients, which constitutes a potential anti-inflammatory mechanism.[66] Furthermore, in a rabbit model of MI, the cardioprotective mechanisms of cangrelor and clopidogrel (measured as decreased infarct size) were shown to involve signal transduction during the reperfusion phase rather than simple inhibition of intravascular coagulation. To further elucidate cangrelor's mechanism of action, the authors used inhibitors for specific signaling pathways. They observed an involvement of adenosine A2B receptors, extracellular signal-regulated kinases (ERKs), Akt, redox signaling, and mitochondrial K_{ATP} channels in the mediation of the protective effect of cangrelor.[60]

The reversibly binding $P2Y_{12}$ antagonist ticagrelor has been reported to increase adenosine levels, which might have an additional protective effect on the microcirculation.[67] Mechanistically, ticagrelor is claimed to increase adenosine levels by inhibiting the equilibrative nucleoside transporter 1, thereby protecting extracellular adenosine from intracellular metabolism.[68] The myocardial adenosine increase provoked by ticagrelor was reported to add to cardioprotection in experimental IRI, for example, in pigs[69] and rats.[70,71] The adenosine-related effects were used in these studies to explain why ticagrelor was superior in its cardioprotective effect compared with other applied $P2Y_{12}$ antagonists, namely, prasugrel and clopidogrel. Interestingly, ticagrelor was shown to enhance adenosine release from human platelets under stirring in vitro.[59]

However, recently published studies also report conflicting results.[65,72] In a clinical study comparing treatment of ST-elevation MI (STEMI) patients with ticagrelor maintenance therapy versus prasugrel, there was no difference observed either in the index of microcirculatory resistance or in infarct size after 1 month. Also, plasma adenosine levels were not increased in patients treated with ticagrelor.[65] Species differences and differences in treatment protocols may be the reason for the still inconclusive data. Furthermore, the notion of the paradoxical role of platelets in IRI makes interpreting data from $P2Y_{12}$ inhibitor studies more complex to interpret.[73] Platelets were shown to exert cardioprotective effects, for example, via release of granule contents or microRNAs, with which $P2Y_{12}$ receptor antagonists may interfere.[73]

Another reported cardioprotective off-target effect of ticagrelor during IRI is the inhibition of the multiprotein platform complex nucleotide-binding oligomerization domain (NOD)-like receptor pyrin domain containing 3 (NLRP3) inflammasome.[59,71,74,75] The NLRP3 inflammasome can induce inflammatory programmed cell death involving caspase-1 activation leading to cardiomyocyte death.[74] A study in diabetic rats suggested that cardioprotection by ticagrelor in rats undergoing experimental IRI was partially attributable to inhibition of the NLRP3 inflammasome. Specifically, in this study, IR-dependent upregulation of NLRP3 mRNA and interleukin (IL)-1β mRNA were attenuated by oral ticagrelor treatment during 3 days before the initiation of myocardial IR.[71] A recently published study by Penna et al has confirmed and extended this finding using isolated hearts from nondiabetic rats. The authors showed that the reduction of infarct size achieved by oral pretreatment of rats with ticagrelor before isolation of hearts and ex vivo IR induction is partially mediated by the inhibition of the NLRP3 inflammasome pathway. Specifically, cardiac protein levels of NLRP3 were significantly decreased which led to reduced caspase-1 activation and less IR-induced cardiac accumulation of active IL-1β. Additionally, with ticagrelor treatment, the authors also observed an upregulation of the reperfusion injury salvage kinase (RISK) pathway and a decrease in IR-induced oxidative stress.[59] When directly administered to the ex vivo heart right before IRI, ticagrelor lacked its cardioprotective effect. Together with the finding that ticagrelor enhances release of sphingosine-1 phosphate (S1P) and adenosine (both cardioprotective substances) from human platelets in vitro, the authors conclude that the target of ticagrelor and thereby mediators of its protective effect are most likely platelets.[59] S1P and adenosine have both been shown to exert protective effects by activation of the cardiac RISK pathway.[76]

Glycoprotein IIb/IIIa Receptor

Activation of GPIIb/IIIa (ITGA2B/ITGB3) and binding to fibrinogen facilitates stable platelet aggregation and thrombus formation. The use of GPIIb/IIIa inhibitors such as abciximab or tirofiban in animal studies has shown this platelet receptor's importance in the development of cardiac damage during MI and IRI. In isolated rat hearts, the infusion with platelets from acute MI patients worsened the myocardial injury induced by experimental IR. Pretreating these platelets with abciximab reduced the platelet-induced damage, as measured by improved markers of myocardial injury (maximal left ventricular [LV] end-diastolic pressure, coronary resistance, lactate dehydrogenase release, infarct size).[58] Interestingly, in addition to interrupting GPIIb/IIIa–fibrinogen binding, abciximab also interferes with other mechanisms of platelet adhesion, for example, via the vitronectin receptor or via leukocyte macrophage-1 antigen (Mac-1).[58,77,78] The effect of GPIIb/IIIa-dependent intracoronary platelet retention on cardiac outcome was also shown in a model of low-flow ischemia followed by reperfusion in isolated guinea pig hearts. In this study, the protective effect of tirofiban when applied during ischemia was attributed to blockade of platelet adherence via GPIIb/IIIa–VWF interaction, as no fibrinogen was present in the experimental system using washed platelets.[79] However, no tirofiban effect on cardiac function was observed when administered during the reperfusion phase.[79] In contrast, a recent study by Kingma showed that tirofiban, when applied at the time of myocardial reperfusion in dogs, led to a reduction of tissue necrosis during reocclusion as well as prolonged occlusion times.[80] Further data supporting a beneficial effect of GPIIb/IIIa inhibition at reperfusion were reported in IRI in rats. There, tirofiban mediated its cardioprotective effect by activating several signaling pathways including activation of protein kinase C (PKC)ε, phosphatidylinositol 3 (PI3) kinase, Akt, p38 mitogen-activated protein kinase (MAPK), p42/44 MAPK, and ERK1/2.[81] It has not been clarified yet whether this effect of tirofiban is directly on the myocardium, or whether the effect was mediated by circulating platelets. Taken together, these studies indicate that GPIIb/IIIa antagonism at the time of reperfusion may limit consequences of IRI, including mechanisms that are distinct from inhibition of platelet adherence.

Glycoprotein VI

The transmembrane protein glycoprotein VI (GPVI) is constitutively associated with the Fc receptor γ (FcRγ) chain, which contains an immunoreceptor tyrosine-based activation motif. This complex is commonly known as the platelet collagen receptor. Platelet activation by collagen through GPVI is mediated via the tyrosine kinase, Syk. It was shown that fibrin can also activate platelets via GPVI which increases thrombin generation and the recruitment of platelets to clots. Consequently, GPVI probably owns a role in thrombus growth and stabilization.[82,83] Interestingly, the platelet GPVI pathway is dispensable for physiological hemostasis but critical for thrombus formation and growth.[84,85] Genetic or pharmacological inhibition of the GPVI receptor leads to improved outcome after myocardial IR with decreased infarct size in mice. Knockout of the FcRγ decreased platelet aggregation and occlusive microthrombi, as well as Syk activation and myeloperoxidase (Mpo) activity in a mouse study applying coronary occlusion and reperfusion.[86] Anti-GPVI treatment with a monoclonal antibody significantly reduced infarct size primarily by improving microperfusion.[87] In contrast to the other studies mentioned, the treatment with the anti-GPVI antibody in this study was accompanied by myocardial hemorrhage in a small subset of mice (two out of nine mice analyzed).[87]

Based on its minor role in hemostasis and overall promising data from animal studies, GPVI was suggested as a promising target for a new class of antiplatelet agents with reduced risk of bleeding complications compared with other common antiplatelet drugs.[37] This is supported by the notion that patients with a GPVI deficiency display only a mild or even no bleeding phenotype.[88] Revacept, a soluble GPVI–Fc fusion protein, that interferes with collagen-mediated platelet adhesion and subsequent aggregation was shown to restore cardiac LV function and reduce infarct size 4 weeks post-IRI in mice.[89] However, in a phase II clinical trial in stable CAD patients undergoing PCI, the intravenous infusion of revacept on top of standard antithrombotic therapy showed no reduction in myocardial injury.[90]

P-Selectin

Activated platelets express P-selectin on their surface. As an adhesion molecule, P-selectin plays an important role in inflammation as it mediates interactions of platelets with leukocytes, for example, in PLCs. P-selectin knockout mice and wild-type (WT) mice transfused with P-selectin knockout platelets show significantly smaller infarct sizes after myocardial IR than WT mice.[91] Similarly, the pharmacological inhibition of platelet P-selectin has beneficial effects on platelet-mediated reperfusion injury after myocardial IR in pigs and rats.[92,93] The harmful effect of platelet P-selectin is thought to be mediated by increasing the inflammatory reaction associated with IRI as P-selectin expression on platelets increases their adherence to the reperfused endothelium and to leukocytes, thereby enhancing leukocyte activation and recruitment. However, when interpreting such data, it must be noted that global P-selectin inhibition strategies also target endothelial P-selectin and thus observed effects are not solely attributable to platelets.[94]

Serotonin

The biogenic amine serotonin (5-hydroxytryptamine) exists in the body in two distinct systems: one as a neurotransmitter in the central nervous system and the other as a hormone in the periphery. Peripheral serotonin is synthesized by tryptophan hydroxylase isoform 1 (Tph1) in the gut and is stored in high concentrations in dense granules of platelets. Platelet activation during acute MI leads to the release of

serotonin.[95,96] The inhibition of serotonin receptors, platelet serotonin uptake, or serotonin production was shown to improve outcome after experimental IRI.[95–98] Mechanistically, serotonin was first believed to indirectly worsen IRI via oxidative stress caused by its enzymatic degradation via mitochondrial monoamine oxidase A that leads to H_2O_2 production.[98] Our group found recently that platelet-derived serotonin induces neutrophil degranulation leading to the release of Mpo and H_2O_2 from neutrophils. Additionally, serotonin enhances the surface expression of CD11b on neutrophils leading to their enhanced recruitment. As a consequence, serotonin worsened inflammation in the infarct tissue and thereby myocardial damage.[96]

The notion that serotonin is elevated after acute MI and associated with IRI[96,99,100] leads to the speculation that this biogenic amine could serve as a biomarker for IRI. In a study by Rieder et al, it was investigated whether serum serotonin has a diagnostic potential in acute MI and to predict IR. However, serum serotonin concentration did not show an association with the severity of CAD or the extent of IRI.[101]

Reactive Oxygen Species

ROSs are generated by cellular oxidative metabolism (e.g., NADPH oxidase and the electron transport chain in mitochondria). These molecules are highly reactive and important for cell signaling. However, an imbalance between ROS production and antioxidant mechanisms results in oxidative stress which is implicated in the pathogenesis of cardiovascular disease.[102,103] Myocardial I/R increases ROS generation. Importantly, in this context platelets are both source and target of ROS.[104] ROSs are involved in the regulation of platelet activation, aggregation, and recruitment.[104] NAD(P)H oxidase isoforms are the main sources of ROS in platelets, followed by COX, xanthine oxidase, and mitochondrial respiration.[104] Early studies showed that platelets are activated by intrinsically generated superoxide anion and hydroxyl radicals after they had undergone anoxia and reoxygenation.[105] Studies on guinea pig hearts revealed that ROS released by platelets can cause IRI independent from mere intracoronary platelet adhesion.[79,103,106] ROS can harm the reperfused myocardium, for example, by damaging membranes and proteins or by opening of the mitochondrial permeability transition pore and subsequently causing apoptosis.[20,106] In the microcirculation, the I/R-related increase in ROS production induces adhesive qualities of the endothelial surface.[41] Furthermore, ROS production activates the complement system in IRI. It was recently shown that the complement C3a receptor expressed on platelets modulates platelet aggregation.[107] Together, the interplay of different sources of ROS and their procoagulant effect on platelets generates a vicious circle that affects different cell types in IRI and contributes to disease progression.[104]

Platelet-Activating Factor

Platelets (as well as leukocytes and endothelial cells) produce the phosphoglyceride platelet-activating factor (PAF) which can exert autocrine and paracrine effects, for example, on cardiomyocytes, endothelial cells, and platelets.[108] Large amounts of PAF are released during IR and can exert negative effects on the heart, including arrhythmias.[76,109,110] PAF mediates these effects through the activation of inflammatory cells like platelets and neutrophils.[108] Inhibiting the PAF receptor was shown to mitigate myocardial IR.[111,112] In an early study by Ko et al, it was demonstrated that administration of a specific PAF receptor antagonist immediately before reperfusion in an intact sheep model reduces myocardial reperfusion injury—an impact which was attributed to reduced reperfusion-dependent platelet and neutrophil activation.[112] In mice, the beneficial effect of PAF receptor deficiency was also shown to be mediated by reduced inflammation, but also by reduced oxidative stress.[111] Furthermore, it is known that PAF stimulates the Na^+/H^+ exchanger isoform 1 (NHE1) in platelets.[113] NHE1 is a membrane protein that removes intracellular H^+, thereby protecting cells from intracellular acidification, and contributes to platelet activation.[113,114] NHE1 inhibition before the onset of myocardial ischemia was shown to reduce infarct size in rats.[115] However, in a clinical trial evaluating the cardioprotective effects of eniporide, a selective inhibitor of the human NHE1, in patients with acute STEMI, no reduction in infarct size or improvement in clinical outcome was observed.[116]

Paradoxically, PAF also exerts cardioprotective effects in picomolar concentrations. The underlying mechanisms involve the activation of the RISK pathway, including protein kinase C, Akt, and nitric oxide synthase.[108,117]

Heterocellular Interactions

Platelets exert many of their effects during IRI in the form of heterocellular interactions, for example, with endothelial cells or leukocytes. A special kind of platelet–leukocyte interactions are PLCs. Platelet–neutrophil (PNCs) and platelet–monocyte complexes (PMCs) are increased in blood from patients suffering from acute MI and their formation is believed to aggravate inflammatory tissue injury.[30,118,119] Mechanistically, the formation of PLC leads to activation of both of the involved cell types and triggers cytokine release as well as adhesion molecule and cell surface receptor exposition.[120,121] PLC formation is also thought to facilitate extravasation of leukocytes.[122,123]

In ex vivo studies applying experimental IRI on isolated hearts, the simultaneous perfusion with both neutrophils and platelets, as compared with perfusion with either platelets or neutrophils, worsened cardiac functions in isolated rat and guinea pig hearts. The harmful effects were shown to be inhibitable by interrupting PNC formation.[124–126] The formation of PLC is initiated by interactions between P-selectin and P-selectin glycoprotein ligand-1 and subsequent interactions between GPIb and Mac-1 that stabilize the intercellular adhesion.[123,127] Several in vivo studies on different species showed that cardiac outcome after IRI can be improved by neutralization of P-selectin,[92,93,128] which resulted in less neutrophil infiltration and platelet–neutrophil adhesion in the infarcted heart tissue.

Conflicting results on the functional relevance of PLC in IRI were reported, too. A study on isolated guinea pig hearts by Seligmann et al did not find an additional effect in the combined presence of platelets and neutrophils over the presence of only one cell type alone, questioning a causative role of PLC in IRI.[129] Furthermore, in a conference abstract, Starz et al reported that mice carrying a selective deletion of platelet P-selectin (P-selectin$^{-/-}$ bone marrow chimeras) display a blunted surge in circulating PLC after induction of experimental myocardial IRI. However, this did not result in any differences in infarct size, tissue inflammation, or long-term ejection fraction in comparison to P-selectin WT. As an explanation for unaltered cardiac outcome, the authors found unaffected leukocyte extravasation in the platelet P-selectin knockout mice as observed via intravital microscopy. These results challenge the prevailing opinion about a pathogenic function of PLC in MI.[130]

During IRI, platelets are triggered by the injured endothelium, but the interaction also works vice versa. Activation and adhesion of platelets is accompanied by the release of numerous proinflammatory and pro-mitogenic substances which alter chemotactic, adhesive, and proteolytic properties of endothelial cells.[27] In the context of IRI, it was shown that inhibiting GPVI-dependent interaction of platelets and endothelial cells improves cardiac function and reduces infarct size in mice. This was due to attenuated platelet degranulation and proinflammatory cytokine release which resulted in less inflammation of the infarcted myocardium.[89] Other mechanisms of platelet-dependent alterations of the endothelial inflammatory phenotype involve platelet CD40 ligand.[131] Furthermore, IR-dependent platelet–neutrophil interactions result in enhanced P-selectin expression on the coronary microvascular endothelium.[124]

Reticulated Platelets

In the existing studies on IR damage, the platelet population was mainly considered as a homogeneous whole. Yet, the specific contribution of platelet subpopulations is still very unclear. Platelets have a limited life span of 8 to 10 days in humans; therefore, they must be constantly renewed by the organism. This process of thrombopoiesis can be strongly stimulated in the context of inflammatory reactions, which leads to an increased proportion of young platelets being found in the circulation.[132,133] These newly formed platelets have been named "reticulated platelets" because they possess remnants of rough endoplasmic reticulum and ribosomes.[134] Reticulated platelets differ from regular platelets by increased RNA content, higher volume and more dense granules, higher levels of surface activation markers, and probably increased reactivity.[135–137] Elevated levels of reticulated platelets have been associated with a higher risk of major adverse cardiovascular events and a higher risk of death in patients with acute coronary syndromes.[138–140] It was shown that reticulated platelets are increased fourfold in patients with STEMI compared with control patients.[132] Interestingly, reticulated platelets were even shown to be especially elevated in STEMI patients compared with other types of acute coronary syndromes.[133] It is not clear from these studies whether reticulated platelets are a mere marker of disease or whether they actively contribute to disease progression. But an indication of the latter is the discovery that reticulated platelets respond worse to the standard antiplatelet agents such as aspirin and thienopyridine $P2Y_{12}$ antagonists.[137] In the respective study, patients undergoing elective PCI were randomized to clopidogrel, low-dose prasugrel, or standard-dose prasugrel. Reticulated platelet levels in the blood of patients (measured as immature platelet count) correlated with impaired platelet response to the ADP receptor antagonist therapy.[137] In addition, similar associations were observed at the time points of peak active metabolite levels of clopidogrel and prasugrel, suggesting that intrinsic properties of reticulated platelets rather than platelet turnover itself contribute to their impaired response to antiplatelet agents.[141] Whether reticulated platelets represent a potential therapeutic target in myocardial IRI remains to be investigated in detail.

Platelets in Tissue Remodeling after IRI

The inflammatory phase of cardiac repair after IRI is followed by a reparative phase which has the task to resolve inflammation and enable (myo)fibroblast proliferation, scar formation, and neovascularization.[142] A finely balanced tissue remodeling process is needed for the recovery of cardiac function after MI.[142] Among the known mediators of fibroblast expansion and trans-differentiation to myofibroblasts are serotonin, transforming growth factor (TGF)-β1, and platelet-derived growth factor.[143] All of these are abundant in platelet granules. Platelet-derived TGF-β1 was shown to contribute to cardiac fibrosis and dysfunction in response to pressure overload.[143] A study from 2014 indicates a potential role of platelet TGF-β1 in acute coronary syndrome as well as a prognostic value of TGF-β1 on clinical outcomes in patients.[144] Another molecule enriched in platelet α-granules is thrombospondin-1 (TSP-1). TSP-1 was shown to negatively regulate myofibroblast density and infiltration into noninfarcted areas.[143] Together, fibroblast activation in cardiac healing must be finely tuned and also spatially and temporally limited to prevent adverse remodeling. Platelets might play a central role in this complex regulatory mechanism.[143]

The regulation of vascularization by platelets is multifaceted as well. Several pro-angiogenic (e.g., stromal cell-derived factor-1α) and antiangiogenic factors (e.g., PF4) are stored in distinct subpopulations of platelet α-granula from where they are released differentially in response to specific agonists.[145,146] A recent publication shows an interplay between platelets and the complement system in angiogenesis. The authors demonstrate that activation of the platelet C5a receptor 1 inhibits collateral artery formation in ischemia-induced revascularization as well as capillary formation and pericyte coverage through the release of PF4.[147] Platelet-dependent modulation of angiogenesis after myocardial IRI seems to be yet another potential pharmaceutical target and is worth to be considered when evaluating antiplatelet strategies in MI.

Conclusion

The mechanisms underlying the pathophysiology of IRI as well as their individual contribution to its progression are to date not fully elucidated. A crucial role of platelets in the course of IRI has been convincingly described in recent years. In vivo and ex vivo studies applying experimental MI in different species showed cardioprotective effects through the inhibition of platelet receptors, adhesion molecules, and certain components of the platelet releasate. However, single-agent approaches targeting platelet-related mechanisms that effectively prevent IRI have not yet entered clinical practice. Therefore, an improved understanding of platelet contributions to IRI is necessary to develop new and effective treatment strategies and further improve the condition of the reperfused myocardium.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

This work is part of SFB1425, funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation #422681845).

References

1 Duerschmied D, Bode C, Ahrens I. Immune functions of platelets. Thromb Haemost 2014;112(04):678–691
2 Antczak AJ, Singh N, Gay SR, Worth RG. IgG-complex stimulated platelets: a source of sCD40L and RANTES in initiation of inflammatory cascade. Cell Immunol 2010;263(01):129–133
3 von Hundelshausen P, Koenen RR, Sack M, et al. Heterophilic interactions of platelet factor 4 and RANTES promote monocyte arrest on endothelium. Blood 2005;105(03):924–930
4 von Hundelshausen P, Weber KS, Huo Y, et al. RANTES deposition by platelets triggers monocyte arrest on inflamed and atherosclerotic endothelium. Circulation 2001;103(13):1772–1777
5 Scheuerer B, Ernst M, Dürrbaum-Landmann I, et al. The CXC-chemokine platelet factor 4 promotes monocyte survival and induces monocyte differentiation into macrophages. Blood 2000;95(04):1158–1166
6 Duerschmied D, Suidan GL, Demers M, et al. Platelet serotonin promotes the recruitment of neutrophils to sites of acute inflammation in mice. Blood 2013;121(06):1008–1015
7 Wagner DD, Frenette PS. The vessel wall and its interactions. Blood 2008;111(11):5271–5281
8 Zarbock A, Polanowska-Grabowska RK, Ley K. Platelet-neutrophil-interactions: linking hemostasis and inflammation. Blood Rev 2007;21(02):99–111
9 Moore KL, Stults NL, Diaz S, et al. Identification of a specific glycoprotein ligand for P-selectin (CD62) on myeloid cells. J Cell Biol 1992;118(02):445–456
10 Ruggeri ZM, Mendolicchio GL. Adhesion mechanisms in platelet function. Circ Res 2007;100(12):1673–1685
11 Coller BS, Peerschke EI, Scudder LE, Sullivan CA. A murine monoclonal antibody that completely blocks the binding of fibrinogen to platelets produces a thrombasthenic-like state in normal platelets and binds to glycoproteins IIb and/or IIIa. J Clin Invest 1983;72(01):325–338
12 Savage B, Almus-Jacobs F, Ruggeri ZM. Specific synergy of multiple substrate-receptor interactions in platelet thrombus formation under flow. Cell 1998;94(05):657–666
13 Ginsberg MH, Forsyth J, Lightsey A, Chediak J, Plow EF. Reduced surface expression and binding of fibronectin by thrombin-stimulated thrombasthenic platelets. J Clin Invest 1983;71(03):619–624
14 Wencel-Drake JD, Painter RG, Zimmerman TS, Ginsberg MH. Ultrastructural localization of human platelet thrombospondin, fibrinogen, fibronectin, and von Willebrand factor in frozen thin section. Blood 1985;65(04):929–938
15 Del Conde I, Crúz MA, Zhang H, López JA, Afshar-Kharghan V. Platelet activation leads to activation and propagation of the complement system. J Exp Med 2005;201(06):871–879
16 Roth GA, Mensah GA, Johnson CO, et al; GBD-NHLBI-JACC Global Burden of Cardiovascular Diseases Writing Group. Global burden of cardiovascular diseases and risk factors, 1990-2019: update from the GBD 2019 study. J Am Coll Cardiol 2020;76(25):2982–3021
17 Prasad A, Stone GW, Holmes DR, Gersh B. Reperfusion injury, microvascular dysfunction, and cardioprotection: the "dark side" of reperfusion. Circulation 2009;120(21):2105–2112
18 Hausenloy DJ, Yellon DM. Myocardial ischemia-reperfusion injury: a neglected therapeutic target. J Clin Invest 2013;123(01):92–100
19 Fröhlich GM, Meier P, White SK, Yellon DM, Hausenloy DJ. Myocardial reperfusion injury: looking beyond primary PCI. Eur Heart J 2013;34(23):1714–1722
20 Yang CF. Clinical manifestations and basic mechanisms of myocardial ischemia/reperfusion injury. Ci Ji Yi Xue Za Zhi 2018;30(04):209–215
21 Zhang W, Chen C, Wang J, Liu L, He Y, Chen Q. Mitophagy in cardiomyocytes and in platelets: a major mechanism of cardioprotection against ischemia/reperfusion injury. Physiology (Bethesda) 2018;33(02):86–98
22 Garcia-Dorado D, Andres-Villarreal M, Ruiz-Meana M, Inserte J, Barba I. Myocardial edema: a translational view. J Mol Cell Cardiol 2012;52(05):931–939
23 Hausenloy DJ, Chilian W, Crea F, et al. The coronary circulation in acute myocardial ischaemia/reperfusion injury: a target for cardioprotection. Cardiovasc Res 2019;115(07):1143–1155
24 Menger MD, Vollmar B. Pathomechanisms of ischemia-reperfusion injury as the basis for novel preventive strategies: is it time for the introduction of pleiotropic compounds? Transplant Proc 2007;39(02):485–488
25 Bonaventura A, Montecucco F, Dallegri F. Cellular recruitment in myocardial ischaemia/reperfusion injury. Eur J Clin Invest 2016;46(06):590–601
26 Frangogiannis NG. The inflammatory response in myocardial injury, repair, and remodelling. Nat Rev Cardiol 2014;11(05):255–265
27 Gawaz M. Role of platelets in coronary thrombosis and reperfusion of ischemic myocardium. Cardiovasc Res 2004;61(03):498–511
28 Golebiewska EM, Poole AW. Platelet secretion: from haemostasis to wound healing and beyond. Blood Rev 2015;29(03):153–162
29 Barrabés JA, Inserte J, Agulló L, Alonso A, Mirabet M, Garcia-Dorado D. Microvascular thrombosis: an exciting but elusive therapeutic target in reperfused acute myocardial infarction. Cardiovasc Hematol Disord Drug Targets 2010;10(04):273–283
30 Köhler D, Straub A, Weissmüller T, et al. Phosphorylation of vasodilator-stimulated phosphoprotein prevents platelet-neutrophil complex formation and dampens myocardial ischemia-reperfusion injury. Circulation 2011;123(22):2579–2590
31 Cameron SJ, Mix DS, Ture SK, et al. Hypoxia and ischemia promote a maladaptive platelet phenotype. Arterioscler Thromb Vasc Biol 2018;38(07):1594–1606
32 Kiouptsi K, Gambaryan S, Walter E, Walter U, Jurk K, Reinhardt C. Hypoxia impairs agonist-induced integrin $\alpha_{IIb}\beta_3$ activation and platelet aggregation. Sci Rep 2017;7(01):7621

33 Braune S, Küpper JH, Jung F. Effect of prostanoids on human platelet function: an overview. Int J Mol Sci 2020;21(23):E9020
34 Allencherril J, Alam M, Levine G, et al. Do we need potent intravenous antiplatelet inhibition at the time of reperfusion during ST-segment elevation myocardial infarction? J Cardiovasc Pharmacol Ther 2019;24(03):215–224
35 Smyth EM. Thromboxane and the thromboxane receptor in cardiovascular disease. Clin Lipidol 2010;5(02):209–219
36 Randomised trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. Lancet 1988;2 (8607):349–360
37 d'Alessandro E, Becker C, Bergmeier W, et al; Scientific Reviewer Committee. Thrombo-inflammation in cardiovascular disease: an expert consensus document from the Third Maastricht Consensus Conference on Thrombosis. Thromb Haemost 2020;120 (04):538–564
38 Loew D, Vinazzer H. Dose-dependent influence of acetylsalicyclic acid on platelet functions and plasmatic coagulation factors. Haemostasis 1976;5(04):239–249
39 Moroz LA. Increased blood fibrinolytic activity after aspirin ingestion. N Engl J Med 1977;296(10):525–529
40 Wu Z, Li X, Li X, Yu L. Effects of aspirin on myocardial ischemia-reperfusion injury in rats through STAT3 signaling pathway. BioMed Res Int 2021;2021:9931885
41 Jackson SP, Darbousset R, Schoenwaelder SM. Thromboinflammation: challenges of therapeutically targeting coagulation and other host defense mechanisms. Blood 2019;133(09):906–918
42 Chambers RC, Laurent GJ. Coagulation cascade proteases and tissue fibrosis. Biochem Soc Trans 2002;30(02):194–200
43 Berlioz BE, Sanghavi D.
;:110–121.Bivalirudin: StatPearls; 2021
44 Lund M, Macwan AS, Tunströmer K, Lindahl TL, Boknäs N. Effects of heparin and bivalirudin on thrombin-induced platelet activation: differential modulation of PAR signaling drives divergent prothrombotic responses. Front Cardiovasc Med 2021;8:717835
45 Anand SX, Kim MC, Kamran M, et al. Comparison of platelet function and morphology in patients undergoing percutaneous coronary intervention receiving bivalirudin versus unfractionated heparin versus clopidogrel pretreatment and bivalirudin. Am J Cardiol 2007;100(03):417–424
46 Erlich JH, Boyle EM, Labriola J, et al. Inhibition of the tissue factor-thrombin pathway limits infarct size after myocardial ischemia-reperfusion injury by reducing inflammation. Am J Pathol 2000; 157(06):1849–1862
47 Kolpakov MA, Rafiq K, Guo X, et al. Protease-activated receptor 4 deficiency offers cardioprotection after acute ischemia reperfusion injury. J Mol Cell Cardiol 2016;90:21–29
48 Kolpakov MA, Guo X, Rafiq K, et al. Loss of protease-activated receptor 4 prevents inflammation resolution and predisposes the heart to cardiac rupture after myocardial infarction. Circulation 2020;142(08):758–775
49 Chaudhary R, Mohananey A, Sharma SP, Singh S, Singh A, Kondur A. Improving outcomes in cardiovascular diseases: a review on vorapaxar. Cardiol Rev 2021;19;. Doi: 10.1097/CRD.0000000000000390
50 Heemskerk JW, Mattheij NJ, Cosemans JM. Platelet-based coagulation: different populations, different functions. J Thromb Haemost 2013;11(01):2–16
51 Ten Cate H, Guzik TJ, Eikelboom J, Spronk HMH. Pleiotropic actions of factor Xa inhibition in cardiovascular prevention: mechanistic insights and implications for anti-thrombotic treatment. Cardiovasc Res 2021;117(09):2030–2044
52 Rocha BML, da Cunha GJL, Aguiar CMT. A narrative review of low-dose rivaroxaban in patients with atherothrombotic cardiovascular disease: vascular protection beyond anticoagulation. Cardiovasc Diagn Ther 2021;11(01):130–141
53 Mega JL, Braunwald E, Wiviott SD, et al; ATLAS ACS 2–TIMI 51 Investigators. Rivaroxaban in patients with a recent acute coronary syndrome. N Engl J Med 2012;366(01):9–19
54 Goto M, Miura S, Suematsu Y, et al. Rivaroxaban, a factor Xa inhibitor, induces the secondary prevention of cardiovascular events after myocardial ischemia reperfusion injury in mice. Int J Cardiol 2016;220:602–607
55 Lorentz CU, Verbout NG, Cao Z, et al. Factor XI contributes to myocardial ischemia-reperfusion injury in mice. Blood Adv 2018;2(02):85–88
56 https://clinicaltrials.gov/
57 Wang K, Zhou X, Huang Y, et al. Adjunctive treatment with ticagrelor, but not clopidogrel, added to tPA enables sustained coronary artery recanalisation with recovery of myocardium perfusion in a canine coronary thrombosis model. Thromb Haemost 2010;104(03):609–617
58 Barrabés JA, Inserte J, Mirabet M, et al. Antagonism of P2Y12 or GPIIb/IIIa receptors reduces platelet-mediated myocardial injury after ischaemia and reperfusion in isolated rat hearts. Thromb Haemost 2010;104(01):128–135
59 Penna C, Aragno M, Cento AS, et al. Ticagrelor conditioning effects are not additive to cardioprotection induced by direct NLRP3 inflammasome inhibition: role of RISK, NLRP3, and redox cascades. Oxid Med Cell Longev 2020;2020:9219825
60 Yang XM, Liu Y, Cui L, et al. Platelet $P2Y^{12}$ blockers confer direct postconditioning-like protection in reperfused rabbit hearts. J Cardiovasc Pharmacol Ther 2013;18(03):251–262
61 Patti G, Bárczi G, Orlic D, et al. Outcome comparison of 600- and 300-mg loading doses of clopidogrel in patients undergoing primary percutaneous coronary intervention for ST-segment elevation myocardial infarction: results from the ARMYDA-6 MI (Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty-Myocardial Infarction) randomized study. J Am Coll Cardiol 2011;58(15):1592–1599
62 Storey RF, Husted S, Harrington RA, et al. Inhibition of platelet aggregation by AZD6140, a reversible oral P2Y12 receptor antagonist, compared with clopidogrel in patients with acute coronary syndromes. J Am Coll Cardiol 2007;50(19):1852–1856
63 Cannon CP, Husted S, Harrington RA, et al; DISPERSE-2 Investigators. Safety, tolerability, and initial efficacy of AZD6140, the first reversible oral adenosine diphosphate receptor antagonist, compared with clopidogrel, in patients with non-ST-segment elevation acute coronary syndrome: primary results of the DISPERSE-2 trial. J Am Coll Cardiol 2007;50(19):1844–1851
64 Cannon CP, Harrington RA, James S, et al; PLATelet inhibition and patient Outcomes Investigators. Comparison of ticagrelor with clopidogrel in patients with a planned invasive strategy for acute coronary syndromes (PLATO): a randomised double-blind study. Lancet 2010;375(9711):283–293
65 van Leeuwen MAH, van der Hoeven NW, Janssens GN, et al. Evaluation of microvascular injury in revascularized patients with ST-segment-elevation myocardial infarction treated with ticagrelor versus prasugrel. Circulation 2019;139(05): 636–646
66 Storey RF, Judge HM, Wilcox RG, Heptinstall S. Inhibition of ADP-induced P-selectin expression and platelet-leukocyte conjugate formation by clopidogrel and the P2Y12 receptor antagonist AR-C69931MX but not aspirin. Thromb Haemost 2002;88(03): 488–494
67 Bonello L, Laine M, Kipson N, et al. Ticagrelor increases adenosine plasma concentration in patients with an acute coronary syndrome. J Am Coll Cardiol 2014;63(09):872–877
68 Cattaneo M, Schulz R, Nylander S. Adenosine-mediated effects of ticagrelor: evidence and potential clinical relevance. J Am Coll Cardiol 2014;63(23):2503–2509
69 Vilahur G, Gutiérrez M, Casani L, et al. Protective effects of ticagrelor on myocardial injury after infarction. Circulation 2016;134(22):1708–1719

70 Ye Y, Birnbaum GD, Perez-Polo JR, Nanhwan MK, Nylander S, Birnbaum Y. Ticagrelor protects the heart against reperfusion injury and improves remodeling after myocardial infarction. Arterioscler Thromb Vasc Biol 2015;35(08):1805–1814

71 Birnbaum Y, Birnbaum GD, Birnbaum I, Nylander S, Ye Y. Ticagrelor and rosuvastatin have additive cardioprotective effects via adenosine. Cardiovasc Drugs Ther 2016;30(06): 539–550

72 Yang XM, Gadde S, Audia JP, Alvarez DF, Downey JM, Cohen MV. Ticagrelor does not protect isolated rat hearts, thus clouding its proposed cardioprotective role through ENT 1 in heart tissue. J Cardiovasc Pharmacol Ther 2019;24(04):371–376

73 Kleinbongard P, Andreadou I, Vilahur G. The platelet paradox of injury versus protection in myocardial infarction - Has it been overlooked? Basic Res Cardiol 2021;116(01):37

74 Mastrocola R, Penna C, Tullio F, et al. Pharmacological inhibition of NLRP3 inflammasome attenuates myocardial ischemia/reperfusion injury by activation of RISK and mitochondrial pathways. Oxid Med Cell Longev 2016;2016:5271251

75 Huang B, Qian Y, Xie S, et al. Ticagrelor inhibits the NLRP3 inflammasome to protect against inflammatory disease independent of the $P2Y_{12}$ signaling pathway. Cell Mol Immunol 2021; 18(05):1278–1289

76 Davidson SM, Andreadou I, Barile L, et al. Circulating blood cells and extracellular vesicles in acute cardioprotection. Cardiovasc Res 2019;115(07):1156–1166

77 Neumann FJ, Zohlnhöfer D, Fakhoury L, Ott I, Gawaz M, Schömig A. Effect of glycoprotein IIb/IIIa receptor blockade on platelet-leukocyte interaction and surface expression of the leukocyte integrin Mac-1 in acute myocardial infarction. J Am Coll Cardiol 1999;34(05):1420–1426

78 Tam SH, Sassoli PM, Jordan RE, Nakada MT. Abciximab (ReoPro, chimeric 7E3 Fab) demonstrates equivalent affinity and functional blockade of glycoprotein IIb/IIIa and alpha(v)beta3 integrins. Circulation 1998;98(11):1085–1091

79 Seligmann C, Simsek Y, Schimmer M, Leitsch T, Bock A, Schultheiss HP. Human thrombocytes are able to induce a myocardial dysfunction in the ischemic and reperfused guinea pig heart mediated by free radicals-role of the GPIIb/IIIa-blocker tirofiban. Life Sci 2002;71(19):2319–2329

80 Kingma JG. Inhibition of Na^+/H^+ exchanger with EMD 87580 does not confer greater cardioprotection beyond preconditioning on ischemia-reperfusion injury in normal dogs. J Cardiovasc Pharmacol Ther 2018;23(03):254–269

81 Chang ST, Yang YT, Chu CM, et al. Protein kinases are involved in the cardioprotective effects activated by platelet glycoprotein IIb/IIIa inhibitor tirofiban at reperfusion in rats in vivo. Eur J Pharmacol 2018;832:33–38

82 Mammadova-Bach E, Ollivier V, Loyau S, et al. Platelet glycoprotein VI binds to polymerized fibrin and promotes thrombin generation. Blood 2015;126(05):683–691

83 Onselaer MB, Hardy AT, Wilson C, et al. Fibrin and D-dimer bind to monomeric GPVI. Blood Adv 2017;1(19):1495–1504

84 Jiang P, Jandrot-Perrus M. New advances in treating thrombotic diseases: GPVI as a platelet drug target. Drug Discov Today 2014; 19(09):1471–1475

85 Stegner D, Nieswandt B. Platelet receptor signaling in thrombus formation. J Mol Med (Berl) 2011;89(02):109–121

86 Takaya N, Katoh Y, Iwabuchi K, et al. Platelets activated by collagen through the immunoreceptor tyrosine-based activation motif in the Fc receptor gamma-chain play a pivotal role in the development of myocardial ischemia-reperfusion injury. J Mol Cell Cardiol 2005;39(06):856–864

87 Pachel C, Mathes D, Arias-Loza AP, et al. Inhibition of platelet GPVI protects against myocardial ischemia-reperfusion injury. Arterioscler Thromb Vasc Biol 2016;36(04):629–635

88 Voors-Pette C, Lebozec K, Dogterom P, et al. Safety and tolerability, pharmacokinetics, and pharmacodynamics of ACT017, an antiplatelet GPVI (glycoprotein VI) Fab. Arterioscler Thromb Vasc Biol 2019;39(05):956–964

89 Schönberger T, Ziegler M, Borst O, et al. The dimeric platelet collagen receptor GPVI-Fc reduces platelet adhesion to activated endothelium and preserves myocardial function after transient ischemia in mice. Am J Physiol Cell Physiol 2012;303(07): C757–C766

90 Mayer K, Hein-Rothweiler R, Schüpke S, et al. Efficacy and safety of revacept, a novel lesion-directed competitive antagonist to platelet glycoprotein VI, in patients undergoing elective percutaneous coronary intervention for stable ischemic heart disease: the randomized, double-blind, placebo-controlled ISAR-PLASTER phase 2 trial. JAMA Cardiol 2021;6(07):753–761

91 Xu Y, Huo Y, Toufektsian MC, et al. Activated platelets contribute importantly to myocardial reperfusion injury. Am J Physiol Heart Circ Physiol 2006;290(02):H692–H699

92 Barrabés JA, Garcia-Dorado D, Mirabet M, et al. Antagonism of selectin function attenuates microvascular platelet deposition and platelet-mediated myocardial injury after transient ischemia. J Am Coll Cardiol 2005;45(02):293–299

93 Oostingh GJ, Pozgajova M, Ludwig RJ, et al. Diminished thrombus formation and alleviation of myocardial infarction and reperfusion injury through antibody- or small-molecule-mediated inhibition of selectin-dependent platelet functions. Haematologica 2007;92(04):502–512

94 Weyrich AS, Ma XY, Lefer DJ, Albertine KH, Lefer AM. In vivo neutralization of P-selectin protects feline heart and endothelium in myocardial ischemia and reperfusion injury. J Clin Invest 1993;91(06):2620–2629

95 Shimizu Y, Minatoguchi S, Hashimoto K, et al. The role of serotonin in ischemic cellular damage and the infarct size-reducing effect of sarpogrelate, a 5-hydroxytryptamine-2 receptor blocker, in rabbit hearts. J Am Coll Cardiol 2002;40(07): 1347–1355

96 Mauler M, Herr N, Schoenichen C, et al. Platelet serotonin aggravates myocardial ischemia/reperfusion injury via neutrophil degranulation. Circulation 2019;139(07):918–931

97 Hohlfeld T, Braun M, Strobach H, Schrör K. Protection of reperfused ischemic pig myocardium by nexopamil, a new combined Ca2+ and serotonin antagonist. J Cardiovasc Pharmacol 1994;23 (06):922–931

98 Bianchi P, Kunduzova O, Masini E, et al. Oxidative stress by monoamine oxidase mediates receptor-independent cardiomyocyte apoptosis by serotonin and postischemic myocardial injury. Circulation 2005;112(21):3297–3305

99 Jin L, Yan XW, Xu SH. [Platelet functions in patients with acute myocardial infarction]. Zhonghua Xin Xue Guan Bing Za Zhi 1989;17(05):275–278, 317

100 Ko YG, Jung JH, Park S, et al. Inflammatory and vasoactive factors in the aspirate from the culprit coronary artery of patients with acute myocardial infarction. Int J Cardiol 2006;112(01):66–71

101 Rieder M, Laumann R, Witsch T, et al. Evaluation of serum serotonin as a biomarker for myocardial infarction and ischemia/reperfusion injury. Appl Sci (Basel) 2020;10(18):6379

102 Aldosari S, Awad M, Harrington EO, Sellke FW, Abid MR. Subcellular reactive oxygen species (ROS) in cardiovascular pathophysiology. Antioxidants 2018;7(01):E14

103 Seligmann C, Schimmer M, Leitsch T, et al. A thrombocyte-induced myocardial dysfunction in the ischemic and reperfused guinea pig heart is mediated by reactive oxygen species. Free Radic Biol Med 2000;29(12):1244–1251

104 Masselli E, Pozzi G, Vaccarezza M, et al. ROS in platelet biology: functional aspects and methodological insights. Int J Mol Sci 2020;21(14):E4866

105 Leo R, Praticò D, Iuliano L, et al. Platelet activation by superoxide anion and hydroxyl radicals intrinsically generated by platelets that had undergone anoxia and then reoxygenated. Circulation 1997;95(04):885–891

106 Seligmann C, Prechtl G, Kusus-Seligmann M, Daniel WG. A myocardial ischemia- and reperfusion-induced injury is mediated by reactive oxygen species released from blood platelets. Platelets 2013;24(01):37–43
107 Sauter RJ, Sauter M, Reis ES, et al. Functional relevance of the anaphylatoxin receptor C3aR for platelet function and arterial thrombus formation marks an intersection point between innate immunity and thrombosis. Circulation 2018;138(16): 1720–1735
108 Penna C, Bassino E, Alloatti G. Platelet activating factor: the good and the bad in the ischemic/reperfused heart. Exp Biol Med (Maywood) 2011;236(04):390–401
109 Montrucchio G, Alloatti G, Camussi G. Role of platelet-activating factor in cardiovascular pathophysiology. Physiol Rev 2000;80 (04):1669–1699
110 Stangl V, Baumann G, Stangl K, Felix SB. Negative inotropic mediators released from the heart after myocardial ischaemia-reperfusion. Cardiovasc Res 2002;53(01):12–30
111 Wang EW, Han YY, Jia XS. PAFR-deficiency alleviates myocardial ischemia/reperfusion injury in mice via suppressing inflammation, oxidative stress and apoptosis. Biochem Biophys Res Commun 2018;495(04):2475–2481
112 Ko W, Lang D, Hawes AS, Zelano JA, Isom OW, Krieger KH. Platelet-activating factor antagonism attenuates platelet and neutrophil activation and reduces myocardial injury during coronary reperfusion. J Surg Res 1993;55(05):504–515
113 Rosskopf D. Sodium-hydrogen exchange and platelet function. J Thromb Thrombolysis 1999;8(01):15–24
114 Chang HB, Gao X, Nepomuceno R, Hu S, Sun D. Na(+)/H(+) exchanger in the regulation of platelet activation and paradoxical effects of cariporide. Exp Neurol 2015;272:11–16
115 Roh HY, Jung IS, Park JW, et al. Cardioprotective effects of [5-(2-methyl-5-fluorophenyl)furan-2-ylcarbonyl]guanidine (KR-32568) in an anesthetized rat model of ischemia and reperfusion heart injury. Pharmacology 2005;75(01):37–44
116 Zeymer U, Suryapranata H, Monassier JP, et al; ESCAMI Investigators. The Na(+)/H(+) exchange inhibitor eniporide as an adjunct to early reperfusion therapy for acute myocardial infarction. Results of the evaluation of the safety and cardioprotective effects of eniporide in acute myocardial infarction (ESCAMI) trial. J Am Coll Cardiol 2001;38(06):1644–1650
117 Penna C, Alloatti G, Cappello S, et al. Platelet-activating factor induces cardioprotection in isolated rat heart akin to ischemic preconditioning: role of phosphoinositide 3-kinase and protein kinase C activation. Am J Physiol Heart Circ Physiol 2005;288 (05):H2512–H2520
118 Ren F, Mu N, Zhang X, et al. Increased platelet-leukocyte aggregates are associated with myocardial no-reflow in patients with ST elevation myocardial infarction. Am J Med Sci 2016;352(03): 261–266
119 Sarma J, Laan CA, Alam S, Jha A, Fox KA, Dransfield I. Increased platelet binding to circulating monocytes in acute coronary syndromes. Circulation 2002;105(18):2166–2171
120 Peters MJ, Dixon G, Kotowicz KT, Hatch DJ, Heyderman RS, Klein NJ. Circulating platelet-neutrophil complexes represent a subpopulation of activated neutrophils primed for adhesion, phagocytosis and intracellular killing. Br J Haematol 1999;106(02): 391–399
121 Huo Y, Schober A, Forlow SB, et al. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. Nat Med 2003;9(01):61–67
122 Maugeri N, Rovere-Querini P, Evangelista V, et al. An intense and short-lasting burst of neutrophil activation differentiates early acute myocardial infarction from systemic inflammatory syndromes. PLoS One 2012;7(06):e39484
123 Page C, Pitchford S. Neutrophil and platelet complexes and their relevance to neutrophil recruitment and activation. Int Immunopharmacol 2013;17(04):1176–1184
124 Lefer AM, Campbell B, Scalia R, Lefer DJ. Synergism between platelets and neutrophils in provoking cardiac dysfunction after ischemia and reperfusion: role of selectins. Circulation 1998;98 (13):1322–1328
125 Götz AK, Zahler S, Stumpf P, Welsch U, Becker BF. Intracoronary formation and retention of micro aggregates of leukocytes and platelets contribute to postischemic myocardial dysfunction. Basic Res Cardiol 2005;100(05):413–421
126 Habazettl H, Hanusch P, Kupatt C. Effects of endothelium/leukocytes/platelet interaction on myocardial ischemia–reperfusion injury. Z Kardiol 2000;89(9, Suppl 9):IX, 92–95
127 Théorêt JF, Bienvenu JG, Kumar A, Merhi Y. P-selectin antagonism with recombinant p-selectin glycoprotein ligand-1 (rPSGL-Ig) inhibits circulating activated platelet binding to neutrophils induced by damaged arterial surfaces. J Pharmacol Exp Ther 2001;298(02):658–664
128 Lefer DJ, Flynn DM, Buda AJ. Effects of a monoclonal antibody directed against P-selectin after myocardial ischemia and reperfusion. Am J Physiol 1996;270(1, Pt 2):H88–H98
129 Seligmann C, Leitsch T, Kusus M, et al. PMN/platelets coinfused in guinea pig hearts Exposed to low-flow ischemia have no additive cardiodepressive effect. J Vasc Res 2003;40(06):501–508
130 Starz C, Härdtner C, Mauler M, et al. Platelet-leukocyte complex formation in myocardial infarction: a marker not a pathogen. Presented at the 87th European Atherosclerosis Society Congress 2019; Maastricht, the Netherlands
131 Henn V, Slupsky JR, Gräfe M, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. Nature 1998;391(6667):591–594
132 Lakkis N, Dokainish H, Abuzahra M, et al. Reticulated platelets in acute coronary syndrome: a marker of platelet activity. J Am Coll Cardiol 2004;44(10):2091–2093
133 Grove EL, Hvas AM, Kristensen SD. Immature platelets in patients with acute coronary syndromes. Thromb Haemost 2009;101 (01):151–156
134 Ts'ao CH. Rough endoplasmic reticulum and ribosomes in blood platelets. Scand J Haematol 1971;8(02):134–140
135 Hoffmann JJ. Reticulated platelets: analytical aspects and clinical utility. Clin Chem Lab Med 2014;52(08):1107–1117
136 Angénieux C, Maître B, Eckly A, Lanza F, Gachet C, de la Salle H. Time-dependent decay of mRNA and ribosomal RNA during platelet aging and its correlation with translation activity. PLoS One 2016;11(01):e0148064
137 Stratz C, Bömicke T, Younas I, et al. Comparison of immature platelet count to established predictors of platelet reactivity during thienopyridine therapy. J Am Coll Cardiol 2016;68(03): 286–293
138 Ibrahim H, Schutt RC, Hannawi B, DeLao T, Barker CM, Kleiman NS. Association of immature platelets with adverse cardiovascular outcomes. J Am Coll Cardiol 2014;64(20):2122–2129
139 Tscharre M, Farhan S, Bruno V, et al. Impact of platelet turnover on long-term adverse cardiovascular outcomes in patients undergoing percutaneous coronary intervention. Eur J Clin Invest 2019;49(09):e13157
140 Cesari F, Marcucci R, Gori AM, et al. Reticulated platelets predict cardiovascular death in acute coronary syndrome patients. Insights from the AMI-Florence 2 Study. Thromb Haemost 2013;109(05):846–853
141 Stratz C, Nührenberg T, Amann M, et al. Impact of reticulated platelets on antiplatelet response to thienopyridines is independent of platelet turnover. Thromb Haemost 2016;116(05): 941–948
142 Prabhu SD, Frangogiannis NG. The biological basis for cardiac repair after myocardial infarction: from inflammation to fibrosis. Circ Res 2016;119(01):91–112
143 Walsh TG, Poole AW. Do platelets promote cardiac recovery after myocardial infarction: roles beyond occlusive ischemic damage. Am J Physiol Heart Circ Physiol 2018;314(05):H1043–H1048

144 Rath D, Chatterjee M, Müller I, et al. Platelet expression of transforming growth factor beta 1 is enhanced and associated with cardiovascular prognosis in patients with acute coronary syndrome. Atherosclerosis 2014;237(02):754–759
145 Chatterjee M, Huang Z, Zhang W, et al. Distinct platelet packaging, release, and surface expression of proangiogenic and antiangiogenic factors on different platelet stimuli. Blood 2011;117 (14):3907–3911
146 Italiano JE Jr, Richardson JL, Patel-Hett S, et al. Angiogenesis is regulated by a novel mechanism: pro- and antiangiogenic proteins are organized into separate platelet alpha granules and differentially released. Blood 2008;111(03):1227–1233
147 Nording H, Baron L, Haberthür D, et al. The C5a/C5a receptor 1 axis controls tissue neovascularization through CXCL4 release from platelets. Nat Commun 2021;12(01):3352

Erratum: This article has been corrected, the Copyright in the bottom line must be © 2022. The Author(s).