

Platelets in Myocardial Ischemia/Reperfusion Injury

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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
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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 (CXCL)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 arrhyth-

mias.^{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 substances²⁹ 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

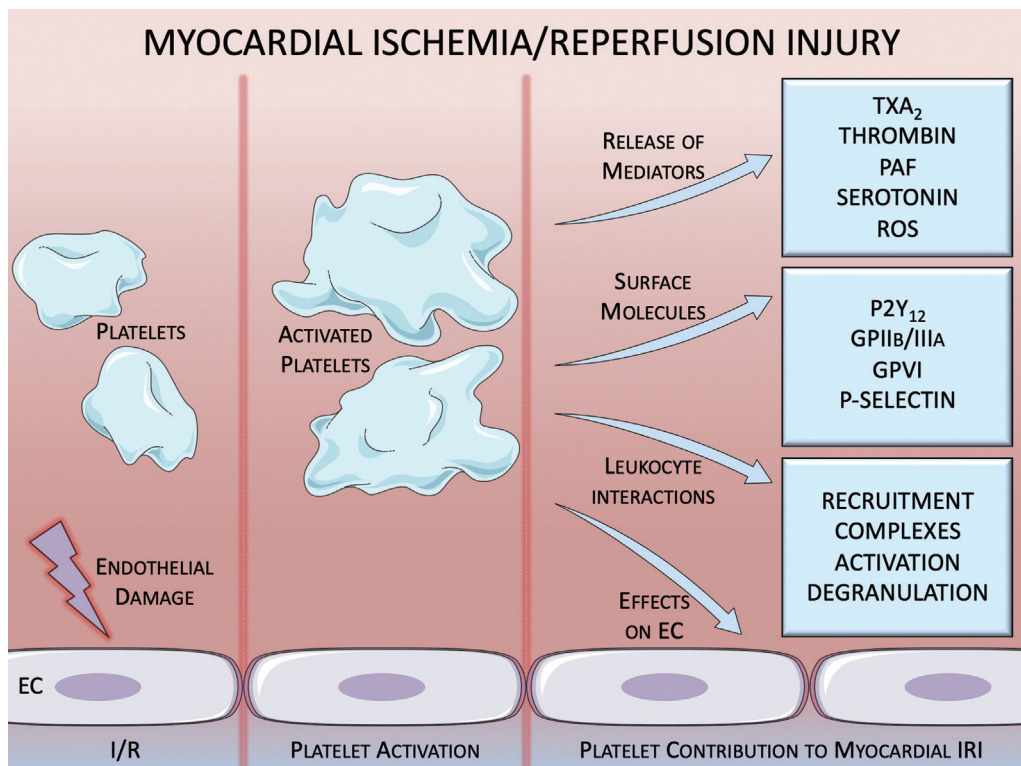


Fig. 1 Platelets in myocardial ischemia/reperfusion injury (IRI). IRI-provoked endothelial damage causes platelet activation. During platelet activation, adhesion proteins, e.g., P-selectin, are expressed or exposed and facilitate interaction with leukocytes, leading to the formation of platelet–leukocyte 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 A₂

Cyclooxygenases (COX) catalyze the conversion of arachidonic acid to prostaglandin G₂ and H₂. This is the prerequisite for subsequent generation of thromboxane A₂ (TXA₂) via thromboxane synthase.³³ Binding of TXA₂ to its receptor on platelets leads to platelet-shape change, inside-out activation of integrins, and degranulation, as well as intracellular Ca²⁺ increase.³³ TXA₂ 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, TXA₂ 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 TXA₂.³⁴ 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 P2Y₁₂ 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 Alto-

gether, 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 activation⁴⁴ 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

P2Y₁₂ Receptor

Activation of the platelet P2Y₁₂ receptor by ADP is important in platelet aggregation and amplifies the platelet response to other agonists. Consequently, inhibition of P2Y₁₂ has an antiaggregatory effect and is beneficial in the treatment of MI. Several P2Y₁₂ 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 P2Y₁₂ inhibitors in dogs,⁵⁷ rats,^{58,59} or rabbits⁶⁰ 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 P2Y₁₂ inhibition.^{61–64} Consequently, P2Y₁₂ 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 P2Y₁₂ inhibition, specific pharmacological (off-target) effects of P2Y₁₂ 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 P2Y₁₂ 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 pigs⁶⁹ 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 P2Y₁₂ 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 P2Y₁₂ 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 P2Y₁₂ 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)-1 β 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-1 β . 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 (FcR γ) 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 he-

mostasis 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 FcR γ 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 H₂O₂ production.⁹⁸ Our group found recently that platelet-derived serotonin induces neutrophil degranulation leading to the release of Mpo and H₂O₂ 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 GPIIb/IIIa-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 P2Y₁₂ 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)- β 1, and platelet-derived growth factor.¹⁴³ All of these are abundant in platelet granules. Platelet-derived TGF- β 1 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- β 1 in acute coronary syndrome as well as a prognostic value of TGF- β 1 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-1 α) 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.

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