Platelets in cardiac ischaemia/reperfusion injury: a promising therapeutic target

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Abstract

Acute myocardial infarction (AMI) is the single leading cause of mortality and morbidity worldwide. A key component of AMI therapy is the timely reopening of occluded vessels to prevent further ischaemic damage to the myocardium. However, reperfusion of the ischaemic myocardium can itself trigger reperfusion injury causing up to 50% of the overall infarct size. In recent years, considerable research has been devoted to understanding the pathogenesis of ischaemia/reperfusion (I/R) injury and platelets have emerged as a major contributing factor. This review summarizes the role of platelets in the pathogenesis of I/R injury and highlights the potential of platelet-directed therapeutics to minimize cardiac I/R injury. Activated platelets infiltrate specifically into the ischaemic/reperfused myocardium and contribute to I/R injury by the formation of microthrombi, enhanced platelet–leucocyte aggregation, and the release of potent vasoconstrictor and pro-inflammatory molecules. This review demonstrates the benefits of platelet inhibition beyond their well-described anti-thrombotic effect and highlights the direct cardioprotective role of anti-platelet drugs. In particular, the inhibition of COX, the P2Y12 receptor and the GPIIb/IIIa receptor has demonstrated the potential to attenuate I/R injury. Moreover, targeting of drug candidates or regenerative cells to the activated platelets accumulated within the ischaemic/reperfused myocardium shows remarkable potential to protect the myocardium from I/R injury. Overall, activated platelets play a key role in the pathogenesis of I/R injury. Their direct inhibition as well as their use as epitopes for site-directed therapy is a unique and promising therapeutic approach for the prevention of I/R injury and ultimately the preservation of cardiac function.

Keyword

Myocardial infarction • Cardiac ischaemia/reperfusion injury • Platelets • Anti-platelet drugs • Targeted therapy

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1. Introduction

Platelets are anucleate, disc-shaped cells averaging 1.5–3.0 μm in diameter. They are generated from megakaryocytes in the bone marrow, circulate in the bloodstream and are cleared in the reticuloendothelial system. The discovery of platelets in 1882 by Giulio Bizzozero and their pivotal role in thrombosis and haemostasis are well known and have been well described.2,3 Resting platelets circulate in the bloodstream until they undergo a rapid shape change at the site of vascular injury, where they roll, adhere, and aggregate to stop bleeding by thrombus formation.4 Extensive, up-to-date research has investigated the potential of anti-platelet therapies to preserve haemostasis.5 Beyond haemostasis and thrombosis, platelets are nowadays characterized as versatile cells directly involved in various physiological and pathophysiological processes such as atherosclerosis,6 angiogenesis, cancer including metastasis7 and inflammation,6 as well as ischaemia/reperfusion (I/R) injury.8

Acute myocardial infarction (AMI) is the single leading cause of death worldwide.9 Timely reperfusion strategies such as pharmacological thrombolysis and percutaneous coronary intervention (PCI) have contributed to substantial improvement in the prognosis of patients suffering AMI. However, paradoxically, restoration of coronary blood flow causes further tissue damage, referred to as myocardial reperfusion injury. In 1960, reperfusion injury was first postulated and characterized by Jennings et al.10 Animal studies showed that reperfusion injury may contribute to the final cardiac infarct size by up to 50%.11 The damage to the myocardium can lead to major clinical consequences such as the no-reflow phenomenon, irreversible and reversible loss of contractile cardiac function, and reperfusion arrhythmias including lethal arrhythmias.12 Furthermore, in up to half of the patients who are successfully treated with primary PCI, coronary microvascular obstruction occurs and these patients are more likely to have a worse outcome.13 Thus, there is clearly an unmet need for therapeutic strategies to limit cardiac I/R injury and to prevent coronary microvascular obstructions.

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The cellular and molecular events underlying myocardial I/R injury are complex and driven by multiple pathways. The key pathways are immune activation and inflammation, endothelial dysfunction, ion accumulation, destabilization of mitochondrial membrane potential, increased formation of reactive oxygen species (ROS), and platelet aggregation and microthrombi formation. This review focuses on platelets in the context of I/R injury and possible platelet-related therapeutic strategies, while other cardioprotective targets are summarized in accompanying reviews in the current Cardiovascular Research spotlight issue. Haematopoietic cells and extracellular vesicles are highlighted by Davidson et al. and Hausenloy et al. discuss the role of the coronary circulation as a cardioprotective target. Two reviews highlight the potential of the immune system as a target for cardioprotection. Andreasou et al. focus on immune cells and Zuurbier et al. describe the cardioprotective potential of novel players and therapeutic opportunities of the innate immune system. The last review focuses on the nervous system as a target for cardioprotection.

**2. Potential platelet-driven pathomechanisms of I/R injury**

We have only started to understand the platelet-associated pathology of I/R injury (Figure 1). Several mechanisms are discussed. First, activated platelets aggregate and form microthrombi in the small cardiac vessels and capillaries, leading to cardiac tissue damage. This is mainly mediated by platelets adhering to reperfused capillary or venular endothelium, or to attached leucocytes, and by releasing vasoconstrictive substances. Platelet glycoproteins (GP) VI and IIb/IIIa play a crucial role in platelet adhesion and aggregation; thus they are crucial for the formation of microthrombi and could serve as potential therapeutic targets. Second, activated platelets within platelet–leucocyte aggregates promote additional pro-inflammatory leucocyte infiltration into the ischaemic/reperfused tissue during the first few days post-I/R. These platelet–leucocyte aggregates are initially formed by interactions between P-selectin and P-selectin glycoprotein ligand-1 (PSGL-1), followed by interactions between GP Ib and Mac-1, stabilizing the cell-to-cell adhesion. Leucocytes with platelets as piggybacks migrate through the endothelium and cause further damage within the cardiac tissue.

Third, activated platelets shed microvesicles (MVs, also referred to as microparticles) and apoptotic bodies, or release exosomes, all of which have the potential to increase inflammation within the ischaemic/reperfused myocardium. MicroRNAs (miRNA) have been shown to play a pivotal role in this process. Several miRNAs are stored and released by platelets, and can exert beneficial effects on cardiac I/R injury, e.g. miRNA-199a, but others can also exert detrimental effects, e.g. miRNA-29. Likewise, there are reports demonstrating that miRNA-126, which was originally described in endothelial cells, can also be expressed and released by platelets, and can increase platelet receptor expression and platelet reactivity. Furthermore, platelets also release thromboxane A2 (TXA2), which is a potent vasoconstrictor, and other vasoconstrictors, leading to endothelial dysfunction. Last, activated platelets also activate the spinal afferent nerves and may lead to chest pain, hypertension, and tachyarrhythmia, and further myocardial damage, by increasing the cardiac oxygen demand.

These potential mechanisms induced by platelet activation after I/R injury lead ultimately to increased infarct size, with the subsequent loss of cardiac function. Current knowledge about the platelet-driven pathology in I/R injury and the respective pharmacological approaches are discussed in more detail in the following paragraphs of this review. Taken together, platelets contribute to cardiac damage as a consequence of I/R injury via these potential mechanisms: formation of microthrombi; platelet–leucocyte aggregation; release of exosomes; shedding of MVs and apoptotic bodies; release of potent vasoconstrictors; and spinal afferent nerve activation.

**3. Role of platelets in I/R injury and their diagnostic potential**

Early animal studies demonstrated that platelets become activated in cardiac I/R injury, infiltrate into the ischaemic myocardium and become trapped, particularly in the marginal zone. Recently, using in vivo models of cardiac I/R injury in mice, various imaging studies provided further evidence that platelets contribute centrally to cardiac reperfusion injury. These studies clearly demonstrated that platelets are activated early during reperfusion after myocardial ischaemia, peaking 2 h post-ischaemic reperfusion and, therefore, are among the first wave of inflammatory cells to infiltrate the infarcted myocardium. Platelet accumulation is strongly correlated with the location of ischaemic and necrotic areas. Furthermore, the number of activated platelets increases in correlation with the duration of ischaemia, supporting the idea that myocardial ischaemia may directly activate platelets. The underlying molecular mechanisms by which ischaemia directly activates platelets is so far unknown and needs to be further investigated. In several studies, platelet activation was not observed in sham-operated animals; this rules out the possibility that the surgical procedure causes platelet activation. Possible mediators of ischaemia-driven platelet activation are activated endothelium in the microcirculation of the myocardium, exposed extracellular matrices, sympathetic response, thromboxane release and release of other platelet-activating cytokines such as stromal cell-derived factor 1 alpha (SDF-1α). Patients with AMI have also been shown to manifest platelet activation and platelets from AMI patients significantly increased cardiac damage when infused into isolated rat hearts. Overall, increasing and convincing evidence for an important role of platelets in experimental cardiac I/R injury has emerged. However, strong clinical data confirming the role of platelets remains to be obtained.

Not only do platelets infiltrate into the ischaemic myocardium following I/R injury, but also the platelets in the periphery change their characteristics due to cardiac I/R injury. RNA-sequencing as well as proteomic studies shed new light on platelet transcriptome and, more specifically, the changes post-I/R injury. In addition to systemic changes in circulating platelets, the proteome of intracoronary platelets in ST elevation myocardial infarction (STEMI) patients showed significant differences compared with peripheral platelets from the same patients. In particular, the integrin subunit αIIb and src kinase–associated phosphoprotein 2 (SKAP2) are upregulated in intracoronary platelets and represent a promising biomarker. Studying the platelet transcriptome is an important method to detect responsive platelet factors and provide mechanistic insights into platelet functions relevant to AMI events, and also allows monitoring for novel platelet-dependent targets to detect or treat I/R injury.
To summarize, platelets accumulate in the ischaemic/reperfused myocardi um for a limited time window. This ‘platelet time window’ may be relatively short but, importantly, it is a consistent, well-defined window. Therefore, through knowing the timeframe of this window, platelet targeting is a promising approach and potentially applicable in clinical practice, as this time window can be very well targeted in the clinical setting of medical or interventional reperfusion.

Interestingly, activated platelets play a major role not only in acute inflammatory response, but also in the excitation of ischaemia-sensitive cardiac spinal afferent nerves, and they contribute to the activation of these afferent nerves during ischaemia, as shown in cats. Activated platelets release exosomes or shed MVs and apoptotic bodies, all of which have the potential to increase inflammation within the ischaemic/reperfused myocardium (top right). Thromboxane A2 (TXA2), also released from activated platelets, is a potent vasoconstrictor, leading to endothelial dysfunction (middle left). Activated platelets aggregate and form microthrombi in the small cardiac vessels and capillaries, leading to cardiac tissue damage (bottom right). Last, activated platelets in platelet–leucocyte aggregates lead to more pro-inflammatory leucocyte infiltration and migration into the ischaemic/reperfused tissue (bottom right). These potential mechanisms characterizing the pathological involvement of platelets after I/R injury ultimately causes increased infarct size and subsequent loss of cardiac function.

A study by Liu et al. showed that platelet accumulation during permanent ischaemia (no reperfusion) is an ongoing process, rather than being limited to the supra-acute phase. Accumulated platelets as well as platelet–leucocyte aggregates become increasingly prominent in the infarcted myocardium within the first 72 h. Platelet accumulation as well as platelet–leucocyte aggregates contribute to myocardial inflammation and wall rupture, and can be significantly inhibited by clopidogrel treatment. Furthermore, anti-platelet therapy with thienopyridines
(clopidogrel or prasugrel) reduces inflammatory cell infiltration into the infarcted myocardium, as well as inflammatory markers such as TNFα and IL-1β.43,44

Therefore, platelet targeting after unsuccessful PCI could be beneficial for a relatively long period of time after AMI.

In summary, platelets are activated early during reperfusion after myocardial ischaemia, and accumulation is strongly correlated with the location of ischaemic and necrotic areas. Platelets play a major role in acute inflammatory response, as well as in the excitation of ischaemia-sensitive cardiac spinal afferent nerves. In the setting of permanent ischaemia, platelet accumulation is an ongoing process and may be a promising target after unsuccessful PCI.

4. Platelets—friend or foe in cardiac I/R injury?

Most reports demonstrate deleterious effects of platelets accumulating in the ischaemic myocardium. Therefore, most current therapeutic strategies aim to inhibit platelet activation to prevent further thrombotic events and inhibit platelet accumulation in the myocardium. However, some studies are investigating the positive role of activated platelets during or post-ischaemia. Studies from Yang et al.45–47 demonstrated that platelets protect the myocardium from I/R injury, mainly due to their release of mediators such as serotonin, TXA2 and adenine nucleotides, which contribute to tissue-protective effects. Gidlöf et al.48 observed that platelets from patients with MI exhibit loss of miRNA-22, -185, -320b, and -423-5p, and demonstrated that activated platelets shed these miRNAs to regulate endothelial cell gene expression. Cheng et al.49 demonstrated improvement in cardiac function and attenuation of cardiac remodelling in infarcted rats when treated with a platelet gel. In concert with these findings, Hargrave et al.50 observed an improvement in left ventricular (LV) function after nanosecond-pulsed platelet-rich plasma (nsPRP) treatment. The authors suggest that the application of nsPRP reduces ROS while providing the antioxidants catalase and superoxide dismutase. These lower ROS levels may contribute to the restored function and partial electrical activity of the left ventricle. Recently, Walsh and Poole51 demonstrated that SDF-1α and transforming growth factor beta (TGF-β), released from α-granules of activated platelets, protect cardiomyocytes from cell death during ischaemia.

Taken together, most studies demonstrated damaging effects of platelet infiltration post-I/R injury; however, there is growing evidence that particular platelet factors are beneficial during ischaemia and after I/R injury.

5. Effects of conditioning on platelets in the ischaemic/reperfused myocardium

Ischaemic pre-conditioning (IPC) describes an experimental technique that exposes a subject to multiple short episodes of ischaemia and reperfusion to prepare the tissue against a subsequent prolonged ischaemic event.52 This phenomenon was first described in 1986 by Murry et al.53 and has since been intensively studied, and protective effects including functional improvement and significantly reduced infarct size have been reported.54,55 Ischaemic post-conditioning describes the process of interrupted myocardial reperfusion by several short-lived episodes of ischaemia56 and remote ischaemic conditioning (RIC) describes the phenomenon of applying non-lethal I/R to a tissue remote from the heart.57 This review focuses on the questions of whether and how these three types of conditioning in coronary ischaemia change platelet activation and accumulation.

In a canine model of coronary I/R injury, IPC decreased platelet activation and formation of both monocyte–platelet and neutrophil–platelet aggregates.58 RIC prior to coronary angiography in a patient cohort with suspected stable angina and double anti-platelet therapy was effective in blocking increases in platelet activation and platelet–monocyte aggregate formation after the procedure. However, PCI did not affect platelet reactivity to adenosine diphosphate (ADP) in this study, which might have been due to the concomitant anti-platelet therapy.59 In the absence of any anti-platelet drug, remote PCI followed by a forearm model of I/R injury showed the same blunting effect on platelet activation measured by platelet–monocyte aggregates compared with the setting where subjects underwent PCI.60 Battipaglia et al.61 showed that RIC protects patients with stable coronary artery disease against an exercise-related increase in platelet reactivity. RIC before radiofrequency catheter ablation for paroxysmal atrial fibrillation inhibits procedure-associated platelet activation and reactivity.62 However, not all studies were able to confirm an effect of RIC on platelet aggregation. A recent study from Riese et al.63 demonstrated in healthy males that RIC had no effect on platelet aggregation and turnover. In concert with this study, IPC in thrombocytopenic rats leads to a reduction in infarct size,64 indicating that preventing platelet activation and aggregation alone is not the underlying mechanism for IPC.

| Author | Year | Study Design | Results |
|--------|------|--------------|---------|
| Zhao   | 2003 | Human study  | IPC decreases platelet activation and aggregation. |
| Hargrave | 2003 | Animal study | Platelets improve LV function after nsPRP treatment. |
| Walsh  | 2003 | Animal study | SDF-1α and TGF-β protect cardiomyocytes in ischaemia. |
| Murry  | 1986 | Animal study | IPC prepares tissue against a subsequent ischaemic event. |

Furthermore, pre-conditioning with volatile anaesthetics like sevoflurane, isoflurane, or xenon may also play a protective role after I/R injury, and seems to influence platelet accumulation. Performing sevoflurane pre- and post-conditioning and using a constant-flow model in guinea pigs prevented post-ischaemic adhesion of leucocytes and platelets;68 this adhesion is known to mediate local and remote tissue damage after I/R injury. Further studies showed that the volatile anaesthetic sevoflurane stabilizes the endothelial glycocalyx, which reduces shedding and cell adhesion, and may ultimately contribute to the cardioprotective effects of volatile anaesthetics.69

Last, beneficial effects with post-conditioning have also been assessed. In 2003 Zhao et al.56 described positive effects of post-conditioning in a canine model of I/R injury, with cardioprotective effects assessed by reduction in infarct size. A recent clinical study of post-conditioning also showed cardioprotective effects assessed by a decrease in oedema and...
an improvement in the myocardial salvage index in STEMI patients. However, this study showed no reduction in infarct size associated with post-conditioning. Several underlying mechanisms are discussed. For example, the inhibition of the mitochondrial permeability transition pore with its beneficial effect on cell survival, stimulation of endogenous ligands such as opioids and adenosine, that act as triggers to stimulate mediators such as protein kinase C, potassium channels, and survival kinases. Adenosine also inhibits platelet activation.

Taken together, in most studies conditioning decreases platelet activation, infiltration, and accumulation when performed prior to cardiac I/R injury, and conditioning represents a promising therapeutic tool to ameliorate myocardial damage caused by I/R injury.

6. Platelet–leucocyte aggregates and their role post-I/R injury

An increased level of platelet–leucocyte aggregates, in particular platelet aggregates with monocytes or neutrophils, has been demonstrated after I/R injury in several animal models and clinical trials. The level of platelet–leucocyte aggregates is also positively associated with the severity of myocardial damage. The receptors responsible for aggregate formation on the platelets are, mainly, for the tethering P-selectin and for the firm interaction the β3-integrin GPIIb/IIIa. Treatment with the GPIIb/IIIa antagonist tirofiban demonstrated a decrease in platelet–leucocyte interaction. The expression of GPIIb/IIIa and subsequent formation of platelet–neutrophil aggregates can be inhibited by phosphorylation of the key regulator cytoskeletal protein vasodilator-stimulated phosphoprotein (VASP). Furthermore, VASP reduces neutrophil-facilitated transendothelial platelet movement. VASP knockout mice showed a significant decrease in blood as well as affected myocardium-infiltrated platelet–neutrophil aggregates, which led ultimately to a decrease in myocardial I/R injury.

In conclusion, platelets contribute indirectly to myocardial I/R injury by increasing platelet–neutrophil aggregates and therefore pro-inflammatory leucocyte adhesion within the post-ischaemic/reperfused myocardium.

7. Roles and changes of platelet MVs and exosomes, platelet-related microRNAs and mean platelet volume after I/R injury

As part of their reaction to activation, platelets release platelet-derived MVs, as well as apoptotic bodies and exosomes. These extracellular vesicles increase after I/R injury and release their cargo: thousands of mediators such as proteins, mRNAs, and microRNAs. MVs are phospholipid-rich particles spanning 100 to 1000 nm and exosomes are small, lipid, bilayer vesicles with a diameter of 50–150 nm. Their role is still unclear, as they appear to have both beneficial as well as detrimental effects. A pro-angiogenic effect of injected platelet MVs was observed in a chronic model of cardiac ischaemia, while their effect in I/R injury needs further study. MVs derived from platelets and monocytes, as well as tissue factor-carrying MVs, are increased in AMI patients. In addition, STEMI patients show higher levels of platelet MVs compared with non-STEMI patients. MVs are related to the extent of myocardial damage. Interestingly, platelet- and monocyte-derived MVs are still increased in patients under anti-platelet therapy. The level of MVs may reflect the platelet activation during ischaemia, and might give information about the severity and prothrombotic stage of the patient. Measurement of the plasma level of MVs and exosomes could be a useful diagnostic tool.

MicroRNAs, specifically when packaged in platelet MVs, have been discussed as possible cardiac biomarkers, as they are significantly increased after cardiac ischaemia. As this review focuses on platelets and platelet-related MVs/microRNA, we do not discuss the potential of general microRNAs as biomarkers here in more detail. However, we summarize current findings on platelet-related microRNAs and their role during AMI. Specific patterns of microRNAs have been shown to be increased in patients with MI. MicroRNAs 126 and 223 have shown high dependency on platelets and are positively correlated with platelet-activation markers. MicroRNA 126 was also showed to modulate ADAM19 and P2Y12 receptor expression, and to alter platelet reactivity. In contrast to platelet MVs, the level of plasma microRNAs is reduced in patients under anti-platelet therapy.

Mean platelet volume (MPV) is related to the reactivity of platelets and is rapidly increased in AMI patients, as well as in mice, post-I/R injury. Greater MPV is associated with impaired reperfusion, adverse cardiovascular events, and high mortality. These larger platelets are most likely released from the spleen, as the spleen represents a large platelet reservoir and splenic platelets are 20–30% larger in size. Newly shed platelets from megakaryocytes are also larger. However, the release of platelets from megakaryocytes requires a long time and so cannot be responsible for the rapid increase in MPV following AMI.

Overall, the levels of platelet-derived MVs, exosomes and certain microRNAs, as well as MPVs, are altered post-I/R injury, and this provides the opportunity to define novel cardiac biomarkers and potential platelet-related therapeutic candidates. However, further research including clarification of their roles as bystanders or pathological agents is needed.

8. Current and future therapeutic interventions to prevent I/R injury

The molecular mechanisms that lead to platelet activation during myocardial I/R injury have not yet been fully identified, but may represent important targets for developing novel and promising diagnostic or therapeutic interventions for I/R injury (Figure 2).

8.1 Cyclooxygenase (COX) inhibitor aspirin

Aspirin is the first-line drug in the management of AMI patients. It blocks COX non-specifically. Platelet function is inhibited by aspirin due to irreversible deactivation of COX-1. COX-1 is an enzyme constitutively expressed in platelets which converts arachidonic acid in prostaglandin H2, followed by conversion into thromboxane A2 (TXA2), a potent promoter of platelet aggregation. Convinving beneficial effects of aspirin in treatment as well as prevention of acute coronary syndrome have been demonstrated in several preclinical studies and large clinical trials. Furthermore, it has been shown that aspirin has effects beyond the well-known platelet-inhibiting effect on COX-1. Aspirin attenuates endothelial dysfunction by blockade of COX-dependent vasconstrictors and has also anti-inflammatory effects in patients with AMI. These beneficial effects clearly outweigh the associated risk of bleeding complications in secondary prevention, but only partially depending on the presence of risk factors in primary prevention.
aspirin has beneficial effects in regards to I/R injury. However, the extent of this potentially beneficial effect and its relation to dosing is not known.

8.2 P2Y12 inhibitors

Together with aspirin, pharmacological inhibition of the P2Y12 receptor on platelets is considered a cornerstone in the management of patients with AMI. Clopidogrel represents the clinically most-used P2Y12 inhibitor; however, the high variability in platelet inhibition between patients and the delayed onset of action are major limitations. The necessary metabolic activation of clopidogrel to its active metabolite may explain its failure to trigger cardioprotection in some preclinical studies.94,95 The newer P2Y12 inhibitors, prasugrel, cangrelor, and ticagrelor, demonstrated greater ability to overcome these limitations96 and have been tested as safe and demonstrated high tolerability.97,98

Interestingly for this review, blocking of the P2Y12 receptor has shown cardioprotective effects through some pleiotropic mechanisms beyond the platelet-inhibition effects. Reduced cardiac infarct size and prevention of reperfusion injury by the P2Y12 inhibitors cangrelor and clopidogrel were noted in a rabbit model with mechanically induced coronary occlusion without the formation of a thrombus.99 Moreover, this study also showed the importance of an immediate-acting P2Y12 blocker. Cangrelor administered at reperfusion reduced infarct size, while delaying the treatment by 10 min after the onset of reperfusion completely eliminated the cardioprotective effect, indicating the importance of administration at the time of recanalization.99 A significant reduction in infarct size was also reported in monkeys when treated with cangrelor.100 The current Platelet Inhibition to Target Reperfusion Injury (PITRI) trial aims to assess whether cangrelor administered prior to reperfusion will reduce AMI size as well as microvascular obstruction in

Figure 2 Platelets as targets for novel therapeutic interventions preventing I/R injury. Platelet receptor-inhibiting approaches show great potential towards amelioration of myocardial damage caused by I/R injury going beyond their well-studied anti-thrombotic effects. Cardioprotective effects have been shown for the COX inhibitor aspirin, P2Y12 receptor inhibitors, GPIIb/IIIa receptor inhibitors, GPVI inhibitors, and P-selectin inhibitors. Abciximab and ticagrelor demonstrate the greatest potential to minimize I/R injury caused cardiac damage. In preclinical studies, site-directed therapy using a single-chain antibody against activated GPIIb/IIIa has shown the greatest promise of minimizing I/R injury by targeted delivery of regenerative cells (Tandem-scFvGPIIb/IIIa-Sca-1) or anti-inflammatory/anti-aggregatory drugs (Targ-CD39). The symbols for preclinical trials, clinical trials and clinical practice next to the drugs indicate the current development state of the described therapeutics.
STEMI patients treated with primary PCI. In a retrospective analysis by Roubille et al. including 88 STEMI patients, clopidogrel treatment resulted in a reduction in final infarct size as assessed by cardiac enzyme release. Ticagrelor treatment in rats after I/R injury showed a reduced myocardial infarct size. This protective effect was described as mainly adenosine and COX-2 dependent. The randomized, double-blind PEGASUS-TIMI 54 trial included over 21,000 patients and demonstrated a reduction in the risk of AMI, stroke, and cardiovascular death. However, ticagrelor treatment was associated with an increased risk of severe bleeding complications. Beneficial effects ofprasugrel were reported in the TRITON-TIMI 38 trial and beneficial effects of cangrelor in the CHAMPION PCI trial. Recent literature compared clopidogrel and ticagrelor, showing that ticagrelor is clinically superior to clopidogrel. Ticagrelor, but not clopidogrel, was reported to reduce fibrinosis in rats 4 weeks after I/R injury. Blocking with ticagrelor is not only reversible and has a faster onset of action, but also reduces necrotic injury and oedema formation in pigs in comparison with clopidogrel. These superior benefits of ticagrelor may be explained by effects beyond its P2Y12 receptor inhibition, as it is hypothesized that ticagrelor also blocks the adenosine re-uptake transporter ENT1 and thus raises tissue adenosine levels. This might explain the clinical benefits seen in the randomized, double-blind Platelet Inhibition and Patient Outcome (PLATO) trial.

In summary, P2Y12 inhibitors have been tested as safe with high tolerability and present clinically promising cardioprotective effects beyond their known anti-thrombotic role, with particularly beneficial tolerability and present clinically promising cardioprotective effects beyond their known anti-thrombotic role, with particularly beneficial effects of ticagrelor and cangrelor.

8.3 Glycoprotein IIb/IIIa inhibitors

Almost two decades ago, the first cardioprotective effects of abciximab (ReoPro), an anti-glycoprotein (GP) IIb/IIIa antibody fragment that inhibits the GPIIb/IIIa receptor, were described. Since then numerous preclinical and clinical trials have investigated the therapeutic potential of GPIIb/IIIa inhibitors in I/R injury. Eptifibatide (Integrilin), a cyclic peptide blocking the GPIIb/IIIa receptor, has been shown in preclinical studies to have similar beneficial effects as abciximab. In 2004, Kunichika et al. studied the effects of platelet inhibition by GPIIb/IIIa inhibition with the peptidomimetic molecule tirofiban (Aggrastat) on microvascular flow after I/R injury in dogs, and demonstrated improved microvascular flow and reduced infarct size. Blocking of the GPIIb/IIIa receptor with the antagonist tirofiban at the onset of reperfusion also reduced the number of infiltrating platelets, as well as post-ischaemic leucocyte recruitment and accumulation in the myocardium. However, the GPIIb/IIIa inhibitor lamifiban showed no preclinical benefits on infarct size after transient coronary occlusion in pigs. Overall, these studies vary in their experimental models as well as animal species, making comparison of the GPIIb/IIIa inhibitors difficult.

The three GPIIb/IIIa inhibitors—abciximab, tirofiban, and eptifibatide—have been approved for clinical use and tested in various clinical trials, with abciximab being the most frequently studied GPIIb/IIIa inhibitor. Several randomized trials demonstrated long-term mortality benefits with abciximab in PCI patients. However, these benefits may be only partly caused by GPIIb/IIIa inhibitory effects, as it was shown that abciximab additionally blocked the leucocyte integrin Mac-1 and therefore also had a direct effect on leucocytes after I/R injury. A clinical trial comparing the beneficial effects of abciximab and eptifibatide in the setting of rescue PCI after failed fibrinolysis demonstrated the superiority of abciximab in comparison to eptifibatide in relation to smaller infarct sizes and improved myocardial perfusion in patients administered abciximab. Despite the beneficial effects of GPIIb/IIIa inhibitors, they are not included in the current standard of care for MI patients. Only a small group of patients, typically with high risk and high thrombotic load, are treated with GPIIb/IIIa inhibitors. This is due to the fact that the newer P2Y12 inhibitors ticagrelor and prasugrel, which are faster and stronger-acting than clopidogrel, have mainly replaced GPIIb/IIIa inhibitors in the acute setting, and also due to the GPIIb/IIIa inhibitor-associated risk of bleeding complications, especially in combination with fibrinolytic drugs.

More recent clinical studies demonstrated a reduction in the bleeding risk, most likely due to improved PCI procedures (e.g. radial access) and the use of weight-adjusted heparin. The safety and efficacy of GPIIb/IIIa inhibitors during PCI have been intensively studied and summarized in a systematic review where the authors assessed 48 studies including over 33,000 patients. In brief, GPIIb/IIIa inhibitors during PCI have been proven to be beneficial, demonstrated by decreases in 30 day mortality, non-fatal MI and urgent revascularization. However, this benefit was associated with an increased risk of severe bleeding complications and the benefit on mortality was lost after 6 months. Activation-specific inhibition of GPIIb/IIIa was shown to prevent impairment of haemostasis in preclinical studies and thus might provide an alternative to the currently approved GPIIb/IIIa inhibitors. Overall, the use of the approved GPIIb/IIIa inhibitors in cardiac reperfusion of patients has been decreasing based on their bleeding side effects and the availability and efficacy of faster-acting P2Y12 inhibitors. Also, specific effects of GPIIb/IIIa inhibitors towards prevention of cardiac I/R injury still have to be determined.

8.4 GPVI inhibitors

Therapeutic targeting of another platelet-adhesion receptor, GPVI, showed benefits post-I/R injury. Schönberger et al. specifically blocked GPVI ligand-binding sites, treating mice with soluble GPVI-Fc (Revacept) after I/R injury, and demonstrated restored cardiac LV function and reduced infarct size 4 weeks post-I/R injury. In line with this study are the findings from Pachel et al. demonstrating a reduction in infarct size as well as a decrease in leucocyte infiltration after I/R injury in mice treated with an anti-GPVI antibody. Furthermore, a study by Yang et al. demonstrated a reduction in infarct size in macaque monkey hearts post-I/R injury when treated with the GPIV antibody OM2.

8.5 GPIb inhibitors

Using a GPIb inhibitor, a recent study demonstrated a reduction in leucocyte infiltration without any effects on infarct size, showing that blockage of GPIb may be of limited benefit in relation to cardioprotection.

8.6 P-selectin

P-selectin was shown to be critical for initial platelet recruitment following I/R injury in a murine model. P-selectin knockout mice showed a decrease in platelet aggregation and infarct size post-I/R injury. In concert with this study, Barrañés et al. demonstrated the potential of the selectin blocker fucoaidan to decrease platelet accumulation in the ischaemic/reperfused myocardium and to attenuate platelet-mediated myocardial damage caused by I/R injury. The P-selectin inhibitor inclucimab has been recently tested in humans and affected neither platelet aggregation nor bleeding time. The SELECT-ACS trial showed great promise in reducing myocardial damage in Non-ST Elevated Myocardial Infarction (NSTEMI) patients when treated prior to PCI.
9. Platelet targeting and platelet activation-inhibiting therapeutics

Accumulated activated platelets represent an ideal target in I/R injury to enrich either drugs or cells specifically in the ischaemic/reperfused cardiac tissue. The activated conformation of GPIb/IIa is an ideal targeting epitope for both diagnostic as well as therapeutic approaches, as it is specific and highly abundant on activated platelets. A single-chain antibody against the active conformation of GPIb/IIa linked to a single-chain antibody (scFv) against a stem cell antigen-1 (Sca-1) receptor showed successful delivery of systemically injected peripheral blood mononuclear cells to the ischaemic/reperfused myocardial tissue, leading to restored cardiac function and decreased infarct size. Furthermore, enriching induced vascular progenitor cells (iVPCs), pre-incubated with the same tandem scFv antibody against the activated GPIb/IIa receptor (Tandem-scFvGPIbIIa-Sca-1), demonstrated significant cardiac benefits 28 days after I/R injury. These iVPC treatment-driven effects were paracrine rather than through direct accumulation and differentiation (engraftment) of iVPCs within the infarcted tissue.

In addition to platelet targeting for cell therapy in cardiac I/R, this unique targeting mechanism can be used for the selective delivery of drugs to the ischaemic/reperfused myocardium. This drug-targeting mechanism allows localized enrichment at the area where the drug effect is needed but allows keeping the systemic concentration low, thereby avoiding the systemic side effects and toxicity present at high concentrations. CD39, an ectonucleotidase whose cardiac genetic overexpression demonstrated less functional loss and a smaller infarct size, is a drug with strong anti-thrombotic and anti-inflammatory effects but severe systemic bleeding side effects. As such, by targeting CD39 to activated platelets (Targ-CD39) the systemic concentration can be lowered to prevent bleeding complications and the beneficial therapeutic effects of CD39 in AML can still be harnessed. In this approach, platelets were not only used as targets for the enrichment of CD39, but also used as an anti-platelet treatment via CD39, as it directly inhibits platelet activation by converting adenosine triphosphate and ADP into adenosine monophosphate, which is then converted into adenosine. The numbers of accumulating platelets were significantly reduced 2 h post-I/R injury, leading to fewer thrombi formations in small cardiac vessels and capillaries, when mice were treated with Targ-CD39. Therefore, Targ-CD39 may also be a means to avoid the coronary microvascular obstruction that occurs in up to half of patients after apparently successful revascularization with PCI.

The therapeutic potential of CD39 targeting has also been investigated in renal I/R injury with a construct that targets CD39 activity to P-selectin, which is expressed on either activated platelets or inflamed endothelial cells, via a 20-amino-acid peptide originating from PSGL-1. Similar to the use of activated GPIb/IIa as a targeting epitope for CD39, P-selectin as a targeting epitope for CD39 demonstrated beneficial effects as seen in histology and improvement of renal function. Overall, the accumulation of activated platelets in ischaemic/reperfused tissue provides unique and specific targeting epitopes, which are the basis for platelet targeting as novel and highly promising approaches for site-directed drug and cell therapy.

10. Conclusions

Platelets are among the first wave of cells infiltrating the myocardium after I/R injury and so provide a unique target for diagnostic imaging and direct functional inhibition, as well as for directing and localizing therapeutics to the area of I/R injury. The precise role of platelets in I/R injury is still not well understood, and the variability in experimental models, different animal species, and different treatment regimens (before, during, or after ischaemia) make the interpretation of their results difficult. However, the crucial involvement of platelets in I/R injury has been consistently described and most studies propose the direct effect of platelets in myocardial damage. Pre-conditioning of ischaemic events performed prior to I/R injury reduces platelet activation and accumulation, and may be a useful therapeutic tool to ameliorate cardiac damage. Activated platelets also contribute indirectly to cardiac I/R injury by increasing platelet-neutrophil aggregates and therefore mediating pro-inflammatory leukocyte infiltration and adhesion after I/R injury. Platelets release exosomes as well as MVs, which themselves release their cargo, causing additional I/R injury. As such, MVs and their specific cargo represent potential biomarkers for I/R injury.

It is generally accepted that an overall reduction in myocardial damage can be achieved by reducing platelet activation and infiltration. The P2Y12 receptor inhibitor ticagrelor demonstrates the greatest potential to ameliorate the myocardial damage caused by I/R injury beyond its well-studied anti-thrombotic effects. Anti-platelet treatment as a standard treatment in AMI patients is an important factor not only in reducing recurring thrombotic events, but also in preventing I/R injury. Activated platelets as targeted epitopes for site-directed therapy have shown in preclinical settings the greatest promise of minimizing I/R injury and ultimate successful translation into clinical practice. Overall, platelets, either through inhibiting their function or as epitopes targeted for site-directed therapy, represent one of the most promising strategies for the prevention of I/R injury to date.

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