



Thomas Jefferson University
Jefferson Digital Commons

Cardeza Foundation for Hematologic Research

Sidney Kimmel Medical College

3-9-2021

Platelets as drivers of ischemia/reperfusion injury after stroke.

Noor F Shaik

Raymond F Regan

Ulhas P Naik

Follow this and additional works at: https://jdc.jefferson.edu/cardeza_foundation

 Part of the [Hematology Commons](#)

[Let us know how access to this document benefits you](#)

This Article is brought to you for free and open access by the Jefferson Digital Commons. The Jefferson Digital Commons is a service of Thomas Jefferson University's [Center for Teaching and Learning \(CTL\)](#). The Commons is a showcase for Jefferson books and journals, peer-reviewed scholarly publications, unique historical collections from the University archives, and teaching tools. The Jefferson Digital Commons allows researchers and interested readers anywhere in the world to learn about and keep up to date with Jefferson scholarship. This article has been accepted for inclusion in Cardeza Foundation for Hematologic Research by an authorized administrator of the Jefferson Digital Commons. For more information, please contact: JeffersonDigitalCommons@jefferson.edu.

Platelets as drivers of ischemia/reperfusion injury after stroke

Noor F. Shaik,¹ Raymond F. Regan,² and Ulhas P. Naik¹

¹Cardeza Foundation for Hematologic Research, Thomas Jefferson University, Philadelphia, PA; and ²Department of Emergency Medicine, School of Medicine, University of Maryland, Baltimore, MD

Ischemic stroke is a leading cause of morbidity and mortality worldwide and, despite reperfusion either via thrombolysis or thrombectomy, stroke patients often suffer from lifelong disabilities. These persistent neurological deficits may be improved by treating the ischemia/reperfusion (I/R) injury that occurs following ischemic stroke. There are currently no approved therapies to treat I/R injury, and thus it is imperative to find new targets to decrease the burden of ischemic stroke and related diseases. Platelets, cell fragments from megakaryocytes, are primarily known for their role in hemostasis. More recently, investigators have studied the nonhemostatic role of platelets in inflammatory pathologies, such as I/R injury after ischemic stroke. In this review, we seek to provide an overview of how I/R can lead to platelet activation and how activated platelets, in turn, can exacerbate I/R injury after stroke. We will also discuss potential mechanisms by which platelets may ameliorate I/R injury.

Introduction

“It is impossible to cure a severe attack of [stroke], and difficult to cure a mild one.” —Hippocrates¹

Strokes are a leading cause of death globally and the leading cause of preventable, long-term disability in the United States.² Over 80% of strokes are primarily ischemic, in which clots occlude blood flow to downstream brain tissue.³ Several clinical trials have established that prompt removal of the clot, either pharmacologically via recombinant tissue plasminogen activator (rt-PA) or by thrombectomy, is beneficial for patients. The 1995 National Institute of Neurological Disorders and Stroke (NINDS) rt-PA Stroke Study was the first to demonstrate that compared with placebo, administration of rt-PA within 3 hours after symptom onset improves functional outcomes in patients 3 months after ischemic stroke.⁴ This trial also highlighted that, although the risk for hemorrhagic transformation (which is bleeding into the brain after clot removal) is 6.4% in patients treated with rt-PA compared with 0.6% of placebo-treated patients, the 90-day mortality is comparable between both groups.⁴

The time window was extended from 3 hours to 4.5 hours following the publication of the 2008 European Cooperative Acute Stroke Study III (ECASS III).⁵ However, considerations of time from stroke onset and risk of hemorrhage have limited the actual number of ischemic stroke patients treated with rt-PA to <10%.⁵⁻⁷ An alternative or complementary treatment to rt-PA in patients with occlusion of proximal large vessels is mechanical thrombectomy, which was found to be clinically beneficial when performed within 6 hours after symptom onset in the Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke in The Netherlands (MR CLEAN).⁸ This time window was further expanded by results of the Diffusion Weighted Imaging or Computerized Tomography Perfusion Assessment with Clinical Mismatch in the Triage of Wake Up and Late Presenting Strokes Undergoing Neurointervention (DAWN) trial, which assessed patients with clinical presentations that were disproportionately more severe than the infarction volume detected on emergent imaging, indicating significant tissue at risk that could be salvaged by reperfusion. In this group,

thrombectomy was associated with better functional outcomes 3 months after the stroke when performed up to 24 hours after symptom onset.⁹

Although it is essential to resupply oxygen and nutrients to injured tissue, subsequent ischemia/reperfusion (I/R) injury can continue propagating cell injury via generation of reactive oxygen species (ROS) and other metabolites that drive inflammation.^{3,10,11} For instance, although the DAWN trial showed a clear clinical benefit in terms of functional outcome and independence 90 days after stroke/thrombectomy compared with standard care, more than half of the patient cohort (51%) still showed persistent functional deficits and lack of functional independence.⁹ And, although NINDS showed an improvement in functional outcome after treatment with rt-PA, the mortality rates between the placebo and rt-PA groups are similar, highlighting the need for treatments to improve survival after stroke.⁴ Therefore, although reperfusion is essential to prevent further cell death and functional decline, the full clinical benefit of reperfusion may not be realized without attention to and treatment of I/R injury. The development of effective reperfusion strategies now presents an urgent need for novel therapies to minimize these secondary injury cascades. Unfortunately, there are presently no approved treatments for I/R injury following strokes.

Antiplatelet therapy with aspirin and/or clopidogrel is already the standard of care for secondary prevention of stroke, supported by several multicenter trials.¹² Conversely, antithrombotic therapies not targeting platelets, including anticoagulation with warfarin, heparin, and low-molecular-weight heparin, are effective only in patients with atrial fibrillation, where they act to prevent atrial clot formation.¹³ Recently, there has been increasing interest in the role of platelets as drivers of inflammation, providing a mechanistic link with I/R injury and potentially identifying novel therapeutic targets.^{14,15}

In this review, we will first discuss the potential mechanisms of I/R-mediated platelet activation and how platelets can worsen reperfusion injury after strokes. Because platelets are renowned for their role in preventing hemorrhage by creating a platelet plug after endothelial injury, we will also discuss potential platelet-based strategies to prevent hemorrhagic transformation. Finally, because the focus of this review is on I/R injury following stroke, emphasis is placed on the filament-induced transient middle cerebral artery (MCA) occlusion (tMCAO) model, which mimics the focal ischemia seen in most strokes and is ideal for studying both reperfusion injury and the potential for hemorrhagic transformation.¹⁶ In this model, the MCA of rodents is occluded unilaterally by inserting a silicon-coated monofilament from the common or external carotid artery to the branch point where the MCA meets the Circle of Willis, causing ischemia by physically preventing blood from entering the MCA; after typically 1 hour, it is removed to allow reperfusion of the brain.

I/R-induced platelet activation

In the following section, we present potential agonists that could activate platelets after I/R (Table 1). There are many others that could play a role in platelet-mediated I/R injury (such as glutamate), but the evidence for these is speculative at this point and they are not discussed here.

I/R-generated ROS and its effect on platelets

One of the first phenomena that occurs in I/R injury is the “oxygen paradox,” whereby ischemia leads to cellular energy failure and

Table 1. List of agonists released after ischemic stroke/reperfusion that could activate platelets

Agonist	Selected references
ROS	11, 17-20
VWF	21-29, 47-49
ADAMTS13	27, 50-52
HMGB1	53-55
ADP	56-58
S1P	59-65
Collagen	25, 66
Thrombin	34, 67-72

ADAMTS13, a disintegrin-like and metalloprotease with thrombospondin type 1 motif number 13; ADP, adenosine 5'-diphosphate; HMGB1, high-mobility group box-1; S1P, sphingosine-1-phosphate; VWF, von Willebrand factor.

a build-up of purinergic degradation compounds, namely hypoxanthine. Then, as blood and oxygen flow back to the region during reperfusion, oxygen fuels xanthine oxidase-mediated conversion of hypoxanthine to xanthine and uric acid, releasing superoxide anion in the process.¹¹ Other sources of oxidative stress include leukocyte reduced nicotinamide adenine dinucleotide phosphate oxidases and electron leak from mitochondria, all of which can contribute to increased ROS following stroke.¹¹ Thus, affected tissues experience an immense amount of oxidative stress caused by the superoxide anion, its byproduct (hydrogen peroxide), and other ROS generated in this process. Furthermore, the brain is very sensitive to ischemia due to its having the highest metabolic activity per unit weight of any organ and lower levels of protective antioxidant enzymes that help mitigate injury from ROS.^{10,11} This leads to an even higher amount of oxidative stress in ischemic strokes compared with I/R in other organs. This I/R-generated ROS can enhance platelet activity as described in the next paragraph.

One of the primary mechanisms for ROS-enhanced platelet activity is quenching of the platelet inhibitor, nitric oxide (NO), by extracellular ROS. NO is synthesized by many cells including platelets, endothelial cells (ECs), and macrophages.¹⁷ How NO potently inhibits platelet adhesion and aggregation to the endothelium is extensively reviewed by others, but in short, NO leads to an increase in cyclic guanosine monophosphate and protein kinase G activation inside platelets, which downregulates calcium signaling and subsequent platelet activation, adhesion, and aggregation.^{17,18} Furthermore, high levels of cyclic guanosine monophosphate in platelets at the edge of a thrombus promote thrombus dissolution in high-shear conditions (such as those in carotid/cerebral vessels), thus preventing complete vessel occlusion.¹⁹ In addition, NO is particularly important for maintaining a healthy endothelium as decreased NO is associated with increased platelet/leukocyte adhesion and vasoconstriction, both of which can lead to capillary no-reflow after reperfusion, worsening the initial ischemic injury.²⁰ Hence, the burst of ROS generated after I/R decreases the bioavailability of NO and subsequently decreases the threshold for platelet activation and thrombus propagation.

Role of VWF axis in I/R injury

von Willebrand factor (VWF) is a glycoprotein (GP) that is synthesized by ECs and stored in/released from their Weibel-Palade bodies; VWF mediates platelet adhesion and subsequent

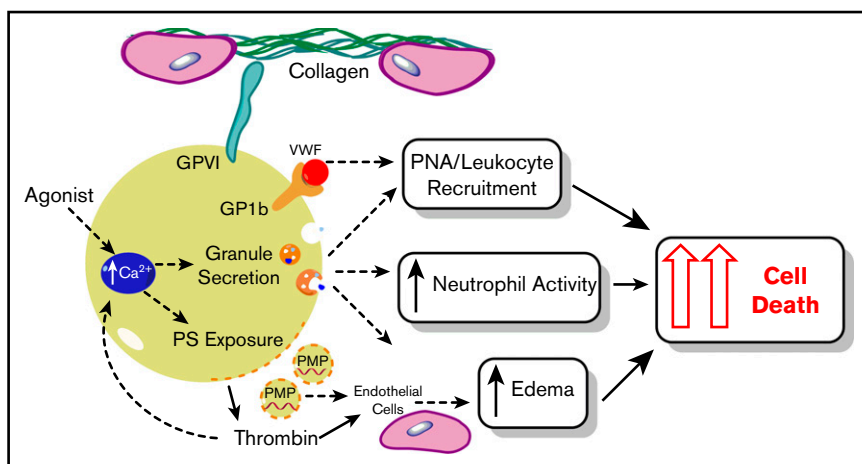


Figure 1. Summary of platelet functions that can affect I/R injury. Following I/R, activated platelets can promote I/R injury by interacting with VWF, binding to exposed subendothelial collagen, secreting granules and recruiting/activating leukocytes, especially neutrophils, exposing phosphatidylserine (PS; which enhances thrombin generation), and releasing platelet microparticles (PMPs; which can affect other cells by delivery of microRNAs) to enhance tissue death. PNA, platelet-neutrophil aggregate.

activation by binding GPIb of the platelet-specific GP Ib-IX-V complex. Both during hypoxia and subsequent reoxygenation, ECs display a pronounced release of VWF in media, just as would occur during I/R.²¹ In addition to hypoxia, uric acid can also stimulate Weibel-Palade body exocytosis in ECs.²² Platelets are an alternate source of VWF because megakaryocytes also synthesize VWF and bequeath it to platelets in their α granules. Although VWF is also released by degranulated platelets, in this section, we are focusing on how VWF released from I/R-injured endothelium can activate platelets. Regardless of the source, VWF is a major player in the progression of I/R injury (Figure 1).^{23,24}

Several groups subsequently pursued the role of VWF in the context of ischemic stroke. Kleinschnitz et al observed that blockade of the VWF receptor GPIb with Fab fragments of the monoclonal antibody p0p/B to GPIb α attenuated tMCAO-induced I/R injury by decreasing both infarct size and neurological deficits.²⁵ Remarkably, this protection persisted even when the Fab fragments to GPIb were injected immediately prior to reperfusion, suggesting a clinically relevant time window.²⁵ Consistent with these observations, 2 groups independently reported that VWF knockout mice were also significantly protected from cerebral I/R injury.^{26,27} By tracking the progression of the infarct and cerebral blood flow using ultra-high-field magnetic resonance imaging in control mice and those treated with the Fab to GPIb at 2 and 24 hours after tMCAO, that is, 1 and 23 hours after reperfusion, Pham et al saw that GPIb blockade increased cerebral blood flow and sustained reperfusion.²⁸ This implies that microthrombi and no-reflow may play a major role in VWF-mediated infarct progression.

Consequently, GPIb blockade was shown to indeed reduce thrombus burden and the amount of occluded cerebral microvessels.²⁹ Furthermore, leukocyte infiltration was also decreased in the treated mice, indicating that VWF is also important for platelet-mediated leukocyte recruitment and subsequent inflammation. Platelet interactions with leukocytes, particularly neutrophils, are known to be key for platelet-mediated I/R injury and have been extensively detailed in the cited excellent reviews, and we refer the reader to these for further information.³⁰⁻³² In short, activated platelets secrete granules and release proinflammatory cytokines that stimulate ECs and leukocytes, leading to edema and heightened neutrophil recruitment and extravasation to the injured tissue.³³⁻³⁹ When recruited to the site of I/R, neutrophils amplify I/R

injury by releasing a burst of ROS as they phagocytose cellular debris.⁴⁰⁻⁴³ Platelets also form complexes with neutrophils known as platelet-neutrophil aggregates (PNAs), which, along with constriction of capillary pericytes, are implicated in capillary no-reflow.⁴⁴⁻⁴⁶ Of note, targeting the A1 domain of VWF in particular (which forms the binding site for GP1b), was recently found to confer protection by decreasing leukocyte recruitment after stroke.⁴⁷ In addition to Fab-mediated GPIb inhibition, a separate group also observed that pharmacologically targeting GPIb with anfibatide similarly protects from stroke-mediated I/R injury.⁴⁸ Moreover, anfibatide treatment reduced vascular permeability and tissue edema.⁴⁹

Another protein in the GPIb-VWF–signaling axis is a disintegrin-like and metalloprotease with thrombospondin type 1 motif number 13 (ADAMTS13). VWF is secreted as large multimers, known as ultra-large VWF, which are subsequently cleaved into smaller fragments by ADAMTS13. Uncleaved ultra-large VWF is highly thrombogenic, and the absence of ADAMTS13 increases cerebral infarction after tMCAO.^{27,50} Infusion of a high dose of recombinant human ADAMTS13 into wild-type (WT) mice reduced infarct size and improved neurological outcomes.²⁷ Mechanistically, absence of ADAMTS13 exacerbates I/R injury by not only leading to a greater accumulation of platelet-VWF-leukocyte aggregates, but also increasing leukocyte infiltration.⁵⁰ Further investigation revealed that ADAMTS13 knockout mice also had elevated levels of high-mobility group box-1 (HMGB1), which is a potential platelet agonist in its own right (discussed in the next paragraph).⁵¹ As with VWF, lack of ADAMTS13 is implicated in both microvascular obstruction and inflammation after stroke.⁵² Thus, not only is GPIb inhibition an attractive target for the amelioration of I/R injury after stroke, but ADAMTS13 infusion is also an exciting alternative because both protect from I/R injury without leading to hemorrhage.²⁷

Proplatelet agonists like DAMPs are released by I/R-injured cells

I/R injury causes damaged cells to release a plethora of molecules, including damage-associated molecular patterns (DAMPs) that induce inflammation.¹¹ One such DAMP is HMGB1, a nonhistone DNA-binding protein, which is rapidly released into the extracellular space following cerebral ischemia and, as aforementioned, also plays a role in the GP1b-VWF–signaling axis after I/R.⁵³ In the

context of platelets, HMGB1 can enhance agonist-induced platelet activation, granule secretion, and thrombus formation via interaction with platelet Toll-like receptor 4.⁵⁴ Intriguingly, platelet activation also resulted in pronounced translocation of HMGB1 from the cytosol to the platelet surface.⁵⁵ Thus, it is possible that platelet HMGB1 may further I/R injury as well. Another DAMP released by necrotic cells is adenosine 5'-diphosphate (ADP), which can bind to platelet receptors P2Y12 and P2Y1. Inhibition of P2Y12 either genetically or pharmacologically protected mice from injury after tMCAO/reperfusion.⁵⁶⁻⁵⁸

Sphingosine-1-phosphate (S1P) is another I/R-associated product that has been implicated in inflammation, and the utility of blocking the sphingolipid pathway in stroke has recently been summarized.⁵⁹ Red blood cells and the vascular endothelium can secrete S1P, and S1P can also activate platelets.⁶⁰⁻⁶² Furthermore, S1P is released by activated platelets and can act as a secondary agonist, and this platelet-released S1P exerts paracrine effects on ECs.⁶² The role of platelet-derived S1P in inflammation has been reviewed.⁶³ Blocking S1PR2 through the inhibitor JTE-013 reduced cerebral I/R injury.⁶⁴ Perhaps some of the protective effect of JTE-013 is due to its inhibition of S1P-induced platelet activation, and because ischemic stroke increases S1P levels; this is a potential topic for future study to tease out the contribution of S1P-induced platelet activation on infarct size and functional outcomes.⁶⁵

Platelet activation following interaction with ECs and collagen

In addition to agonist-induced platelet activation, platelets can also bind to stimulated endothelium just minutes after reperfusion via cell-adhesion molecules (ICAM, VCAM), CD40/CD40L interactions, EC $\alpha_V\beta_3$ to platelet $\alpha_{IIb}\beta_3$ via fibrinogen, and E-selectin/P-selectin upregulated on I/R-injured ECs.^{32,35} Others have reviewed these mechanisms, and we refer the reader to these excellent reviews for details.^{15,32} Of note, platelet adhesion to the I/R-stimulated endothelium was particularly affected by blocking P-selectin interactions compared with ICAM/ $\alpha_{IIb}\beta_3$ interactions after tMCAO.³⁹ Although platelets can directly bind to the I/R-stimulated endothelium, electron microscopy demonstrated that reperfusion worsened endothelial denudation in the rat brain as compared with ischemia alone.⁶⁶ Because endothelial denudation exposes the subendothelial matrix, this means that, after I/R, platelets also come in contact with one of their most potent endogenous agonists, collagen (Figure 1). As such, targeting the platelet collagen receptor GPVI with Fab fragments of JAQ1 monoclonal antibody after stroke decreased infarct volume.²⁵

Finally, thrombin is a serine protease that not only potently activates platelets but also induces endothelial permeability.³⁴ Plasma samples from ischemic stroke patients revealed an increased level of thrombin-antithrombin complexes (a measure of thrombin activation) after atherothrombotic infarcts.⁶⁷ Along those lines, lower concentrations of thrombin-antithrombin correlated with better recanalization following rt-PA treatment in patients.⁶⁸ Studies in mice showed that both interfering with the coagulation cascade (by targeting tissue factor, factor XII, or kallikrein) and directly inhibiting thrombin (via argatroban) decreased infarct size after ischemic stroke.⁶⁹⁻⁷²

Platelet-mediated I/R injury

As depicted in Figure 1, the next section will focus on how various platelet-mediated activities can affect I/R injury; namely, we will look

at integrin $\alpha_{IIb}\beta_3$, platelet-secreted products, phosphatidylserine (PS) exposure, and microparticle (MP) formation and how these may impact tissue death after ischemic stroke/reperfusion.

$\alpha_{IIb}\beta_3$ inhibition has little effect on I/R injury

Platelet integrin $\alpha_{IIb}\beta_3$ is not only essential for platelet aggregation, but it also mediates PNA formation (via neutrophil $\alpha_M\beta_2$) and facilitates leukocyte recruitment and platelet-EC interaction (through EC $\alpha_V\beta_3$).^{14,30,35} Hence, $\alpha_{IIb}\beta_3$ was identified as a primary therapeutic target for I/R injury. There are already many $\alpha_{IIb}\beta_3$ inhibitors on the market, including the chimeric monoclonal antibody abciximab and a synthetic molecule, tirofiban; however, these may be "too effective" at inhibiting platelet activity because associated bleeding complications have limited their use as antiplatelet drugs. Indeed, multiple groups have shown that $\alpha_{IIb}\beta_3$ blockade has limited or no effect on infarct size, whereas it actually increases intracerebral hemorrhage and mortality.^{25,49,73} Thus, although $\alpha_{IIb}\beta_3$ was once considered a promising target because of the significantly increased risk of hemorrhagic transformation with $\alpha_{IIb}\beta_3$ inhibitors, other less potent drugs may be worth evaluating.

Platelet-secreted products have widespread effects on I/R injury

Platelets have 3 main types of granules, α/δ granules, and lysosomes, which fuse with the plasma membrane to release their contents following platelet activation (Figure 1).⁷⁴ But before looking at the role that some of these granule contents play in cerebral I/R injury, we will first discuss the overall effect of decreasing platelet granules or their secretion. In humans, gray platelet syndrome is a rare bleeding disorder presenting with few to no α granules; this was replicated in mice by deleting the neurobeachin-like 2 (*Nbeal2*) gene.⁷⁵ Loss of *Nbeal2* in hematopoietic cells led to a 50% reduction in infarct size.⁷⁵ Similarly, Munc13-4, a protein product of the *Unc13d* gene, is involved in secretion of α and δ granules, and its absence leads to complete abrogation of δ -granule secretion and partial abrogation (~60%) of α -granule secretion.⁷⁶ The absence of Munc13-4 in *Unc13d* knockout mice also revealed dramatic protection after tMCAO.⁷⁶

Regarding the many constituents of platelet granules, several (like ADP) are secondary platelet agonists and increase platelet activation in a paracrine/autocrine manner, whereas others (like platelet factor 4 and β -thromboglobulin) are proinflammatory cytokines that serve to not only recruit leukocytes, but also activate ECs. In addition to the EC/platelet P-selectin interactions mentioned in "Platelet activation following interaction with ECs and collagen," platelet P-selectin, which is brought to the surface after platelet α -granule secretion, interacts with neutrophil P-selectin glycoprotein ligand-1 to form PNAs and to recruit neutrophils to the injury.¹⁴ Additionally, P-selectin may even potentiate neutrophil extracellular trap formation in I/R, and target neutrophil extracellular traps via DNase-improved outcomes in tMCAO mice.⁷⁷ Very recently, Denorme et al found that preventing platelet necrosis (by targeting cyclophilin D, which is also important for PS exposure, discussed in the next paragraph) not only reduced the amount of P-selectin-positive platelets after tMCAO, but also decreased PNAs and infarct size.⁷⁸

Effects of PS-mediated thrombin generation and MP release on I/R injury

Under resting conditions, anionic PS is on the inner leaflet of the platelet membrane. Following platelet activation, PS is rapidly

exposed via the activity of platelet scramblases and indirectly facilitates the conversion of prothrombin to thrombin by recruiting the coagulation proteins Xa and Va.⁷⁹ As mentioned herein, thrombin can both amplify platelet activation and increase edema formation by inducing endothelial permeability; as such, inhibiting platelet-mediated thrombin formation by decreasing PS exposure would be expected to be protective in ischemic stroke. However, infarct size was not reduced by a platelet-specific knockout of the scramblase transmembrane protein 16F (which is important for PS exposure), despite reduction in PS exposure, thrombin generation, and thrombosis.^{79,80}

Another phenomenon that occurs following platelet activation is MP release. Because MPs bud off platelets (and other cell types, but we are focusing on platelet-derived MPs), they often carry along granule and cytosolic components. An additional mechanism by which platelet MPs (PMPs) can interact with other cells is through the transfer of microRNAs (miRs) (Figure 1).^{81,82} For example, postischemia treatment with a mir-181a antagomir, which would bind to and silence endogenous miR-181a (which is present in platelets), reduced infarct size and improved functional recovery in mice.⁸³ Other miRs present in platelets that could also affect I/R injury include miR-106b, miR-93, miR-223, and miR-656 (which was shown to be significantly associated with stroke risk in patients).^{82,84-87} Although no studies have yet been reported that specifically studied the question of whether PMP-delivered miRs can affect stroke size and subsequent behavioral recovery, this is certainly an interesting line of study.

Platelet-derived factors may reduce hemorrhagic transformation

Although an abundance of data establishes the role of platelets as drivers of I/R injury after stroke, there is a small body of research that supports the view that platelet activity is important, if not necessary, to protect from I/R injury and aid in recovery following ischemic stroke.⁸⁸ This is not surprising because, in addition to proinflammatory cytokines, activated platelets can also release anti-inflammatory cytokines like interleukin 10.⁸⁹ Platelets also store both proangiogenic

and antiangiogenic factors that may regulate recovery.⁹⁰ From the research already presented, blockade of the platelet integrin $\alpha_{IIb}\beta_3$ showed limited/no effect on infarct size, while increasing intracerebral hemorrhage and mortality following tMCAO.^{25,49,73}

Additionally, whereas the individual *Nbeal2* and *Unc13d* knockouts reduced I/R injury, when tMCAO was performed in *Nbeal2/Unc13d* double knockout mice (DKO), half of the DKO mice died, as opposed to no mortality in the WT mice; this high mortality rate was reversed when WT platelets were transfused into DKO mice.⁹¹ The increase in death was attributed to massive intracerebral hemorrhages.⁹¹ Intriguingly, of the DKO mice that did survive tMCAO, they showed a statistically significant decrease in infarct volume, just as the individual knockouts had.⁹¹ Therefore, some contents of platelet granules are necessary to prevent hemorrhage and reduce mortality after reperfusion. This is further supported by the recent report that transfusion of resting, but not degranulated, platelets ameliorated hemorrhage after tPA administration in tMCAO mice.⁹² Hence, the intracerebral hemorrhage seen in both of these settings clearly demonstrates that some level of “normal” platelet function is necessary to prevent exacerbation of I/R injury, especially for preventing hemorrhages.

Furthermore, both platelet lysate and the delivery of PMPs conferred protection from ischemic stroke.⁹³⁻⁹⁶ Regarding platelet secreted/released factors that may help with central nervous system repair after stroke, one of the prime candidates is brain-derived neurotrophic factor (BDNF). First, activation of platelet thrombin receptors (PAR1 and PAR4) led to release of BDNF from platelets.⁹⁷ Immunoelectron microscopy showed BDNF to be stored in both α granules and in the cytosol of platelets, and it was released within 5 minutes after thrombin stimulation.⁹⁷ Use of BDNF in stroke promoted repair by increasing neurogenesis, oligodendrogenesis, and remyelination of injured white matter.⁹⁸⁻¹⁰⁰ It is important to note that although this pathway has not been established in stroke models, it seems a worthy topic for further investigation. In summary, not only can antiplatelet therapy at the time of reperfusion (eg, targeting the GPIIb-VWF axis) attenuate I/R

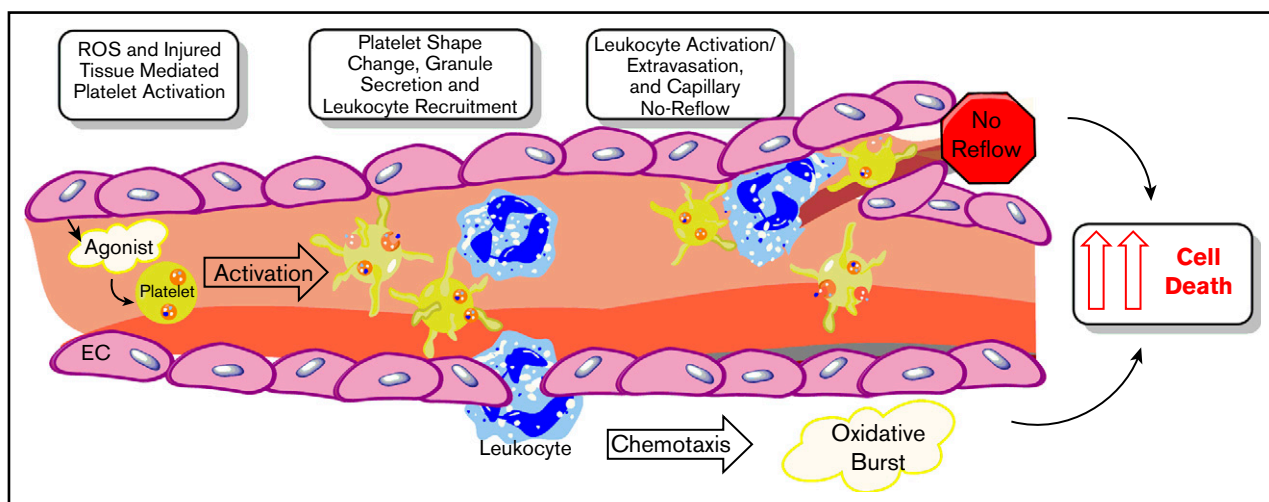


Figure 2. Overview of the processes by which activated platelets mediate I/R injury. Following interaction with injured ECs, ROS, and factors released by I/R-injured cells, platelets promote I/R injury by secreting granules and interacting with leukocytes. This includes enhancing leukocyte extravasation, oxidative burst, and capillary no-reflow (which includes PNAs, fibrin deposition, and other cellular debris; only PNAs are shown for simplicity).

injury after stroke, but subsequent treatment with neurotrophic factors found in platelets, such as BDNF, could also help facilitate neuronal repair and expedite recovery after ischemic stroke.

Concluding remarks

Platelets play a prominent role in reperfusion injury following ischemia: going through the expanse of literature that clearly demonstrates a plethora of ways in which platelets drive inflammation after I/R, to a small but intriguing body of research that suggests platelets may also promote recovery after ischemic stroke. Thus, by now, it is clear that platelets are not just involved in the clot that initiates ischemic stroke, but, rather, platelets are major players in the progression of I/R injury. Upon reperfusion, platelets can respond to I/R-generated agonists such as ROS, HMGB1, and VWF. In addition to molecules secreted by injured cells, platelets can also bind to and be activated by injured endothelium and subendothelial matrix components exposed after I/R injury. These multitudinous mechanisms converge on platelet activation, which includes platelet granule secretion, PS exposure, and PMP release. Ultimately, these lead to EC activation, leukocyte recruitment, and capillary no-reflow, which worsen tissue death and expand the size of the infarct (Figure 2).

Once platelets are activated, the most promising targets to treat I/R injury are those that prevent platelet adhesion and activation, not those that inhibit downstream effects such as platelet aggregation or thrombin generation.^{25,49,73,79} This is likely because rapid secretion of platelet granule contents seems to be the most critical mechanism by which platelets exacerbate I/R injury after stroke.^{75,76} These platelet-secreted products cannot only activate ECs and induce vascular permeability, but they can also potentially recruit and induce leukocytes.³² Because complete blockade of granule secretion can lead to hemorrhage, the better and more efficacious

strategy seems to be in attenuating I/R-mediated platelet activation in the first place.⁹¹ But, although reducing platelet activity is a promising approach to decrease cell death and functional deficits, it is important to keep in mind that platelet integrin function and granule secretion should be at least partially intact to prevent hemorrhagic transformation. In summary, although thrombolytic therapies have improved the functional outcome of stroke patients compared with patients without reperfusion, many stroke patients suffer from lifelong disabilities, reaffirming Hippocrates' point even today.² Thus, in order to achieve the full clinical benefit of reperfusion, identifying new targets for treating I/R injury is essential to decreasing stroke-related death and disability.

Acknowledgments

The authors thank Kalyan Golla for helping edit this manuscript.

This work was supported by National Institutes of Health, National Heart, Lung, and Blood Institute grant 2R01 HL113188-07 (U.P.N.), American Heart Association Grant-in-Aid Award #13GRNT16380023 (U.P.N.), an American Society of Hematology Bridge Grant (U.P.N.), and National Institutes of Health, National Institute of Neurological Disorders and Stroke grant R01 NS095205 (R.F.R.).

Authorship

Contribution: N.F.S., R.F.R., and U.P.N. wrote the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

ORCID profiles: N.F.S., 0000-0002-9336-4238; U.P.N., 0000-0001-7556-444X.

Correspondence: Ulhas P. Naik, Thomas Jefferson University, JAH 394, 1020 Locust St, Philadelphia, PA 19107; e-mail: ulhas.naik@jefferson.edu.

References

1. Clarke E. Apoplexy in the Hippocratic writings. *Bull Hist Med.* 1963;37:301-314.
2. Virani SS, Alonso A, Benjamin EJ, et al; American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics-2020 update: a report from the American Heart Association. *Circulation.* 2020;141(9):e139-e596.
3. Pan J, Konstas AA, Bateman B, Ortolano GA, Pile-Spellman J. Reperfusion injury following cerebral ischemia: pathophysiology, MR imaging, and potential therapies. *Neuroradiology.* 2007;49(2):93-102.
4. National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med.* 1995;333(24):1581-1587.
5. Hacke W, Kaste M, Bluhmki E, et al; ECASS Investigators. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med.* 2008;359(13):1317-1329.
6. Lees KR, Bluhmki E, von Kummer R, et al; ECASS, ATLANTIS, NINDS and EPITHET rt-PA Study Group. Time to treatment with intravenous alteplase and outcome in stroke: an updated pooled analysis of ECASS, ATLANTIS, NINDS, and EPITHET trials. *Lancet.* 2010;375(9727):1695-1703.
7. Jickling GC, Liu D, Stamova B, et al. Hemorrhagic transformation after ischemic stroke in animals and humans. *J Cereb Blood Flow Metab.* 2014;34(2):185-199.
8. Berkhemer OA, Fransen PS, Beumer D, et al; MR CLEAN Investigators. A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med.* 2015;372(1):11-20.
9. Nogueira RG, Jadhav AP, Haussen DC, et al; DAWN Trial Investigators. Thrombectomy 6 to 24 hours after stroke with a mismatch between deficit and infarct. *N Engl J Med.* 2018;378(1):11-21.
10. Saver JL. Time is brain—quantified. *Stroke.* 2006;37(1):263-266.
11. Kalogeris T, Baines CP, Krenz M, Korthis RJ. Ischemia/reperfusion. *Compr Physiol.* 2016;7(1):113-170.
12. Hackam DG, Spence JD. Antiplatelet therapy in ischemic stroke and transient ischemic attack. *Stroke.* 2019;50(3):773-778.
13. Hankey GJ, Berge E, Sandercock P. Anticoagulants versus antiplatelet agents for acute ischemic stroke. *Stroke.* 2003;34(6):1571-1572.

14. Sreeramkumar V, Adrover JM, Ballesteros I, et al. Neutrophils scan for activated platelets to initiate inflammation. *Science*. 2014;346(6214):1234-1238.
15. Ed Rainger G, Chimen M, Harrison MJ, et al. The role of platelets in the recruitment of leukocytes during vascular disease. *Platelets*. 2015;26(6):507-520.
16. Fluri F, Schuhmann MK, Kleinschnitz C. Animal models of ischemic stroke and their application in clinical research. *Drug Des Devel Ther*. 2015;9:3445-3454.
17. Bladowski M, Gawrys J, Gajecki D, Szahidewicz-Krupska E, Sawicz-Bladowska A, Doroszko A. Role of the platelets and nitric oxide biotransformation in ischemic stroke: a translative review from bench to bedside. *Oxid Med Cell Longev*. 2020;2020:2979260.
18. Wang GR, Zhu Y, Halushka PV, Lincoln TM, Mendelsohn ME. Mechanism of platelet inhibition by nitric oxide: in vivo phosphorylation of thromboxane receptor by cyclic GMP-dependent protein kinase. *Proc Natl Acad Sci USA*. 1998;95(9):4888-4893.
19. Wen L, Feil S, Wolters M, et al. A shear-dependent NO-cGMP-cGKI cascade in platelets acts as an auto-regulatory brake of thrombosis [published correction appears in *Nat Commun*. 2018;9(1):4969]. *Nat Commun*. 2018;9(1):4301.
20. Zhu J, Song W, Li L, Fan X. Endothelial nitric oxide synthase: a potential therapeutic target for cerebrovascular diseases. *Mol Brain*. 2016;9:30.
21. Pinsky DJ, Naka Y, Liao H, et al. Hypoxia-induced exocytosis of endothelial cell Weibel-Palade bodies. A mechanism for rapid neutrophil recruitment after cardiac preservation. *J Clin Invest*. 1996;97(2):493-500.
22. Kuo MC, Patschan D, Patschan S, et al. Ischemia-induced exocytosis of Weibel-Palade bodies mobilizes stem cells. *J Am Soc Nephrol*. 2008;19(12):2321-2330.
23. Verhene S, Denorme F, Libbrecht S, et al. Platelet-derived VWF is not essential for normal thrombosis and hemostasis but fosters ischemic stroke injury in mice. *Blood*. 2015;126(14):1715-1722.
24. Dhanesha N, Prakash P, Doddapattar P, et al. Endothelial cell-derived von Willebrand factor is the major determinant that mediates von Willebrand factor-dependent acute ischemic stroke by promoting postischemic thrombo-inflammation. *Arterioscler Thromb Vasc Biol*. 2016;36(9):1829-1837.
25. Kleinschnitz C, Pozgajova M, Pham M, Bendszus M, Nieswandt B, Stoll G. Targeting platelets in acute experimental stroke: impact of glycoprotein Ib, VI, and IIb/IIIa blockade on infarct size, functional outcome, and intracranial bleeding. *Circulation*. 2007;115(17):2323-2330.
26. Kleinschnitz C, De Meyer SF, Schwarz T, et al. Deficiency of von Willebrand factor protects mice from ischemic stroke. *Blood*. 2009;113(15):3600-3603.
27. Zhao BQ, Chauhan AK, Canault M, et al. von Willebrand factor-cleaving protease ADAMTS13 reduces ischemic brain injury in experimental stroke. *Blood*. 2009;114(15):3329-3334.
28. Pham M, Helluy X, Kleinschnitz C, et al. Sustained reperfusion after blockade of glycoprotein-receptor-Ib in focal cerebral ischemia: an MRI study at 17.6 Tesla. *PLoS One*. 2011;6(4):e18386.
29. Schuhmann MK, Guthmann J, Stoll G, Nieswandt B, Kraft P, Kleinschnitz C. Blocking of platelet glycoprotein receptor Ib reduces "thrombo-inflammation" in mice with acute ischemic stroke. *J Neuroinflammation*. 2017;14(1):18.
30. Page C, Pitchford S. Neutrophil and platelet complexes and their relevance to neutrophil recruitment and activation. *Int Immunopharmacol*. 2013;17(4):1176-1184.
31. Lisman T. Platelet-neutrophil interactions as drivers of inflammatory and thrombotic disease. *Cell Tissue Res*. 2018;371(3):567-576.
32. Gawaz M. Role of platelets in coronary thrombosis and reperfusion of ischemic myocardium. *Cardiovasc Res*. 2004;61(3):498-511.
33. Shan LY, Li JZ, Zu LY, et al. Platelet-derived microparticles are implicated in remote ischemia conditioning in a rat model of cerebral infarction. *CNS Neurosci Ther*. 2013;19(12):917-925.
34. Rabiet MJ, Plantier JL, Rival Y, Genoux Y, Lampugnani MG, Dejana E. Thrombin-induced increase in endothelial permeability is associated with changes in cell-to-cell junction organization. *Arterioscler Thromb Vasc Biol*. 1996;16(3):488-496.
35. Bombeli T, Schwartz BR, Harlan JM. Adhesion of activated platelets to endothelial cells: evidence for a GPIIb/IIIa-dependent bridging mechanism and novel roles for endothelial intercellular adhesion molecule 1 (ICAM-1), alpha5beta3 integrin, and GPIbalpha. *J Exp Med*. 1998;187(3):329-339.
36. Lindemann S, Tolley ND, Dixon DA, et al. Activated platelets mediate inflammatory signaling by regulated interleukin 1beta synthesis. *J Cell Biol*. 2001;154(3):485-490.
37. Rosenberg GA, Cunningham LA, Wallace J, et al. Immunohistochemistry of matrix metalloproteinases in reperfusion injury to rat brain: activation of MMP-9 linked to stromelysin-1 and microglia in cell cultures. *Brain Res*. 2001;893(1-2):104-112.
38. Kim GW, Kondo T, Noshita N, Chan PH. Manganese superoxide dismutase deficiency exacerbates cerebral infarction after focal cerebral ischemia/reperfusion in mice: implications for the production and role of superoxide radicals. *Stroke*. 2002;33(3):809-815.
39. Ishikawa M, Cooper D, Arumugam TV, Zhang JH, Nanda A, Granger DN. Platelet-leukocyte-endothelial cell interactions after middle cerebral artery occlusion and reperfusion. *J Cereb Blood Flow Metab*. 2004;24(8):907-915.
40. Jy W, Mao WW, Horstman L, Tao J, Ahn YS. Platelet microparticles bind, activate and aggregate neutrophils in vitro. *Blood Cells Mol Dis*. 1995;21(3):217-231.
41. 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.
42. Litt MR, Jeremy RW, Weisman HF, Winkelstein JA, Becker LC. Neutrophil depletion limited to reperfusion reduces myocardial infarct size after 90 minutes of ischemia. Evidence for neutrophil-mediated reperfusion injury. *Circulation*. 1989;80(6):1816-1827.
43. Mauler M, Seyfert J, Haenel D, et al. Platelet-neutrophil complex formation-a detailed in vitro analysis of murine and human blood samples. *J Leukoc Biol*. 2016;99(5):781-789.

44. Franks ZG, Campbell RA, Weyrich AS, Rondina MT. Platelet-leukocyte interactions link inflammatory and thromboembolic events in ischemic stroke. *Ann N Y Acad Sci.* 2010;1207:11-17.
45. del Zoppo GJ, Schmid-Schönbein GW, Mori E, Copeland BR, Chang CM. Polymorphonuclear leukocytes occlude capillaries following middle cerebral artery occlusion and reperfusion in baboons. *Stroke.* 1991;22(10):1276-1283.
46. Hall CN, Reynell C, Gesslein B, et al. Capillary pericytes regulate cerebral blood flow in health and disease. *Nature.* 2014;508(7494):55-60.
47. Denorme F, Martinod K, Vandenbulcke A, et al. The von Willebrand factor A1 domain mediates thromboinflammation, aggravating ischemic stroke outcome in mice [published online ahead of print 27 February 2020]. *Haematologica.* doi:10.3324/haematol.2019.241042.
48. Li TT, Fan ML, Hou SX, et al. A novel snake venom-derived GPIb antagonist, anfibatide, protects mice from acute experimental ischaemic stroke and reperfusion injury. *Br J Pharmacol.* 2015;172(15):3904-3916.
49. Chen C, Li T, Zhao Y, et al. Platelet glycoprotein receptor 1b blockade ameliorates experimental cerebral ischemia-reperfusion injury by strengthening the blood-brain barrier function and anti-thrombo-inflammatory property. *Brain Behav Immun.* 2018;69:255-263.
50. Fujioka M, Hayakawa K, Mishima K, et al. ADAMTS13 gene deletion aggravates ischemic brain damage: a possible neuroprotective role of ADAMTS13 by ameliorating postischemic hypoperfusion. *Blood.* 2010;115(8):1650-1653.
51. Fujioka M, Nakano T, Hayakawa K, et al. ADAMTS13 gene deletion enhances plasma high-mobility group box1 elevation and neuroinflammation in brain ischemia-reperfusion injury. *Neurol Sci.* 2012;33(5):1107-1115.
52. Khan MM, Motto DG, Lentz SR, Chauhan AK. ADAMTS13 reduces VWF-mediated acute inflammation following focal cerebral ischemia in mice. *J Thromb Haemost.* 2012;10(8):1665-1671.
53. Kim JB, Lim CM, Yu YM, Lee JK. Induction and subcellular localization of high-mobility group box-1 (HMGB1) in the postischemic rat brain. *J Neurosci Res.* 2008;86(5):1125-1131.
54. Vogel S, Bodenstern R, Chen Q, et al. Platelet-derived HMGB1 is a critical mediator of thrombosis. *J Clin Invest.* 2015;125(12):4638-4654.
55. Maugeri N, Franchini S, Campana L, et al. Circulating platelets as a source of the damage-associated molecular pattern HMGB1 in patients with systemic sclerosis. *Autoimmunity.* 2012;45(8):584-587.
56. Chin Y, Kishi M, Sekino M, et al. Involvement of glial P2Y₁ receptors in cognitive deficit after focal cerebral stroke in a rodent model. *J Neuroinflammation.* 2013;10:95.
57. Yamauchi K, Imai T, Shimazawa M, lwama T, Hara H. Effects of ticagrelor in a mouse model of ischemic stroke. *Sci Rep.* 2017;7(1):12088.
58. Devanathan V, Hagedorn I, Köhler D, et al. Platelet Gi protein Gαi2 is an essential mediator of thrombo-inflammatory organ damage in mice. *Proc Natl Acad Sci USA.* 2015;112(20):6491-6496.
59. Sun N, Keep RF, Hua Y, Xi G. Critical role of the sphingolipid pathway in stroke: a review of current utility and potential therapeutic targets. *Transl Stroke Res.* 2016;7(5):420-438.
60. Venkataraman K, Lee YM, Michaud J, et al. Vascular endothelium as a contributor of plasma sphingosine 1-phosphate. *Circ Res.* 2008;102(6):669-676.
61. Hänel P, Andréani P, Gräler MH. Erythrocytes store and release sphingosine 1-phosphate in blood. *FASEB J.* 2007;21(4):1202-1209.
62. Randriamboavonjy V, Badenhop K, Schmidt H, Geisslinger G, Fisslthaler B, Fleming I. The S1P(2) receptor expressed in human platelets is linked to the RhoA-Rho kinase pathway and is down regulated in type 2 diabetes. *Basic Res Cardiol.* 2009;104(3):333-340.
63. Vito CD, Hadi LA, Navone SE, et al. Platelet-derived sphingosine-1-phosphate and inflammation: from basic mechanisms to clinical implications. *Platelets.* 2016;27(5):393-401.
64. Kim GS, Yang L, Zhang G, et al. Critical role of sphingosine-1-phosphate receptor-2 in the disruption of cerebrovascular integrity in experimental stroke. *Nat Commun.* 2015;6:7893.
65. Salas-Perdomo A, Miró-Mur F, Gallizioli M, et al. Role of the S1P pathway and inhibition by fingolimod in preventing hemorrhagic transformation after stroke. *Sci Rep.* 2019;9(1):8309.
66. Nishigaya K, Yoshida Y, Sasuga M, Nukui H, Ooneda G. Effect of recirculation on exacerbation of ischemic vascular lesions in rat brain. *Stroke.* 1991;22(5):635-642.
67. Kataoka S, Hirose G, Hori A, Shirakawa T, Saigan T. Activation of thrombosis and fibrinolysis following brain infarction. *J Neurol Sci.* 2000;181(1-2):82-88.
68. Fernandez-Cadenas I, Mendioroz M, Munuera J, et al. Lower concentrations of thrombin-antithrombin complex (TAT) correlate to higher recanalisation rates among ischaemic stroke patients treated with t-PA. *Thromb Haemost.* 2009;102(4):759-764.
69. Niiro M, Nagayama T, Yunoue S, Obara S, Hirano H. Changes in tissue factor and the effects of tissue factor pathway inhibitor on transient focal cerebral ischemia in rats. *Thromb Res.* 2008;122(2):247-255.
70. Kleinschnitz C, Stoll G, Bendzus M, et al. Targeting coagulation factor XII provides protection from pathological thrombosis in cerebral ischemia without interfering with hemostasis. *J Exp Med.* 2006;203(3):513-518.
71. Göb E, Reymann S, Langhauser F, et al. Blocking of plasma kallikrein ameliorates stroke by reducing thromboinflammation. *Ann Neurol.* 2015;77(5):784-803.
72. Morris DC, Zhang L, Zhang ZG, et al. Extension of the therapeutic window for recombinant tissue plasminogen activator with argatroban in a rat model of embolic stroke. *Stroke.* 2001;32(11):2635-2640.
73. Choudhri TF, Hoh BL, Zerwes HG, et al. Reduced microvascular thrombosis and improved outcome in acute murine stroke by inhibiting GP IIb/IIIa receptor-mediated platelet aggregation. *J Clin Invest.* 1998;102(7):1301-1310.

74. Li Z, Delaney MK, O'Brien KA, Du X. Signaling during platelet adhesion and activation. *Arterioscler Thromb Vasc Biol.* 2010;30(12):2341-2349.
75. Deppermann C, Cherpokova D, Nurden P, et al. Gray platelet syndrome and defective thrombo-inflammation in Nbeal2-deficient mice. *J Clin Invest.* 2013;123(8):3331-3342.
76. Stegner D, Deppermann C, Kraft P, et al. Munc13-4-mediated secretion is essential for infarct progression but not intracranial hemostasis in acute stroke. *J Thromb Haemost.* 2013;11(7):1430-1433.
77. De Meyer SF, Suidan GL, Fuchs TA, Monestier M, Wagner DD. Extracellular chromatin is an important mediator of ischemic stroke in mice. *Arterioscler Thromb Vasc Biol.* 2012;32(8):1884-1891.
78. Denorme F, Manne BK, Portier I, et al. Platelet necrosis mediates ischemic stroke outcome in mice. *Blood.* 2020;135(6):429-440.
79. Fujii T, Sakata A, Nishimura S, Eto K, Nagata S. TMEM16F is required for phosphatidylserine exposure and microparticle release in activated mouse platelets. *Proc Natl Acad Sci USA.* 2015;112(41):12800-12805.
80. Baig AA, Haining EJ, Geuss E, et al. TMEM16F-mediated platelet membrane phospholipid scrambling is critical for hemostasis and thrombosis but not thromboinflammation in mice-Brief Report. *Arterioscler Thromb Vasc Biol.* 2016;36(11):2152-2157.
81. Edelstein LC. The role of platelet microvesicles in intercellular communication. *Platelets.* 2017;28(3):222-227.
82. Pan Y, Liang H, Liu H, et al. Platelet-secreted microRNA-223 promotes endothelial cell apoptosis induced by advanced glycation end products via targeting the insulin-like growth factor 1 receptor. *J Immunol.* 2014;192(1):437-446.
83. Xu LJ, Ouyang YB, Xiong X, Stary CM, Giffard RG. Post-stroke treatment with miR-181 antagomir reduces injury and improves long-term behavioral recovery in mice after focal cerebral ischemia. *Exp Neurol.* 2015;264:1-7.
84. Wang P, Liang X, Lu Y, Zhao X, Liang J. MicroRNA-93 downregulation ameliorates cerebral ischemic injury through the Nrf2/HO-1 defense pathway. *Neurochem Res.* 2016;41(10):2627-2635.
85. Li P, Shen M, Gao F, et al. An antagomir to microRNA-106b-5p ameliorates cerebral ischemia and reperfusion injury in rats via inhibiting apoptosis and oxidative stress. *Mol Neurobiol.* 2017;54(4):2901-2921.
86. Mick E, Shah R, Tanriverdi K, et al. Stroke and circulating extracellular RNAs. *Stroke.* 2017;48(4):828-834.
87. Simon LM, Edelstein LC, Nagalla S, et al. Human platelet microRNA-mRNA networks associated with age and gender revealed by integrated plateletomics. *Blood.* 2014;123(16):e37-e45.
88. Rivera FJ, Kazanis I, Ghevaert C, Aigner L. Beyond clotting: a role of platelets in CNS repair? *Front Cell Neurosci.* 2016;9:511.
89. Linke B, Schreiber Y, Picard-Willems B, et al. Activated platelets induce an anti-inflammatory response of monocytes/macrophages through cross-regulation of PGE2 and cytokines. *Mediators Inflamm.* 2017;2017:1463216.
90. Walsh TG, Metharom P, Berndt MC. The functional role of platelets in the regulation of angiogenesis. *Platelets.* 2015;26(3):199-211.
91. Deppermann C, Kraft P, Volz J, et al. Platelet secretion is crucial to prevent bleeding in the ischemic brain but not in the inflamed skin or lung in mice. *Blood.* 2017;129(12):1702-1706.
92. Luo H, Wei L, Lu L, et al. Transfusion of resting platelets reduces brain hemorrhage after intracerebral hemorrhage and tPA-induced hemorrhage after cerebral ischemia. *Front Neurosci.* 2019;13:338.
93. Kazanis I, Feichtner M, Lange S, et al. Lesion-induced accumulation of platelets promotes survival of adult neural stem/progenitor cells. *Exp Neurol.* 2015; 269:75-89.
94. Hayon Y, Dashevsky O, Shai E, Varon D, Leker RR. Platelet lysates stimulate angiogenesis, neurogenesis and neuroprotection after stroke. *Thromb Haemost.* 2013;110(2):323-330.
95. Hayon Y, Shai E, Varon D, Leker RR. The role of platelets and their microparticles in rehabilitation of ischemic brain tissue. *CNS Neurol Disord Drug Targets.* 2012;11(7):921-925.
96. Hayon Y, Dashevsky O, Shai E, Brill A, Varon D, Leker RR. Platelet microparticles induce angiogenesis and neurogenesis after cerebral ischemia. *Curr Neurovasc Res.* 2012;9(3):185-192.
97. Tamura S, Suzuki H, Hirowatari Y, et al. Release reaction of brain-derived neurotrophic factor (BDNF) through PAR1 activation and its two distinct pools in human platelets. *Thromb Res.* 2011;128(5):e55-e61.
98. Rodríguez-Frutos B, Otero-Ortega L, Ramos-Cejudo J, et al. Enhanced brain-derived neurotrophic factor delivery by ultrasound and microbubbles promotes white matter repair after stroke. *Biomaterials.* 2016;100:41-52.
99. Ramos-Cejudo J, Gutiérrez-Fernández M, Otero-Ortega L, et al. Brain-derived neurotrophic factor administration mediated oligodendrocyte differentiation and myelin formation in subcortical ischemic stroke. *Stroke.* 2015;46(1):221-228.
100. Schäbitz WR, Steigleder T, Cooper-Kuhn CM, et al. Intravenous brain-derived neurotrophic factor enhances poststroke sensorimotor recovery and stimulates neurogenesis. *Stroke.* 2007;38(7):2165-2172.