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Platelets Are at the Nexus of Vascular Diseases

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Platelets are important actors of cardiovascular diseases (CVD). Current antiplatelet drugs that inhibit platelet aggregation have been shown to be effective in CVD treatment. However, the management of bleeding complications is still an issue in vascular diseases. While platelets can act individually, they interact with vascular cells and leukocytes at sites of vascular injury and inflammation. The main goal remains to better understand platelet mechanisms in thrombo-inflammatory diseases and provide new lines of safe treatments. Beyond their role in hemostasis and thrombosis, recent studies have reported the role of several aspects of platelet functions in CVD progression. In this review, we will provide a comprehensive overview of platelet mechanisms involved in several vascular diseases.

Keywords: vascular diseases, leukocytes, inflammation, platelets activation, thrombosis

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Since their first description by Osler (1) and later by Bizzozero (2), platelets have gained a lot of attention in many biological processes. Among the earliest evidence that platelets are crucial for human hemostasis, is based on platelet transfusions in thrombocytopenic patients that can restore hemostatic function. Platelets play a critical role in hemostasis by maintaining the integrity of blood vessels. They provide the first line of defense following injury, forming thrombi that patch-up damaged endothelial tissue and thereby play an indispensable role in hemostasis. However, dysregulated platelet activation can lead to thrombosis, myocardial infarction and stroke. Platelets are also involved in the development of atherosclerosis in coronary or carotid arteries, which is commonly the trigger for thrombosis. Plaque rupture is a common cause of arterial thrombosis and leads to the exposure of thrombogenic components to the flowing blood. The current dogma is that arterial thrombi are composed of aggregated platelets and venous thrombi are enriched in fibrin. However, this view has been challenged with landmark works by several groups on the role of platelets in venous thrombosis (3, 4). The molecular mechanism of thrombus formation has been extensively reviewed in detail elsewhere (5). Here we will provide a brief description of the role of platelets in clot formation and discuss their implication in vascular diseases.

PLATELET MECHANISMS IN ARTERIAL THROMBOSIS AND VASCULAR INFLAMMATION

At sites of vascular injury, the subendothelial extracellular matrix (ECM) is exposed to the blood, to which platelets promptly adhere in order to limit hemorrhage and promote tissue healing. This matrix contains several adhesive macromolecules such as collagen, von Willebrand factor (vWF), laminin, fibronectin and thrombospondin, all of which serve as ligands for different platelet surface receptors. Among these subendothelial substrates, the thrombogenic fibrillar collagens type I and III are by far the most potent mediators of platelet adhesion due to their strong platelet activating potential and affinity for vWF. Transient adhesion of platelets (tethering) depends largely on

vWF and its receptor, the GPIb-V-IX complex. Platelets express multiple surface receptors that directly or indirectly interact with collagen, among which integrin $\alpha 2\beta 1$ (GPIa/IIa) and the Ig-like receptor glycoprotein VI (GPVI) are the most important ones. Integrin $\alpha 2\beta 1$ predominantly mediates adhesion, whereas GPVI is the collagen-activated receptor in platelets. GPVI is a transmembrane protein of 62 kDa and belongs to the family of immunoreceptor tyrosine-based activation motif (ITAM) receptors. GPVI binds to the Fc receptor γ chain (FcR γ) which triggers the signaling cascade. Activation of platelets by GPVI-collagen interactions leads to the activation of phospholipase C and the subsequent mobilization of the second messengers calcium (Ca^{2+}) and diacylglycerol (DAG). DAG is critical for protein kinase C (PKC) activation, a key event in platelet granule release and integrin activation (6). Ca^{2+} regulates various adhesive platelet responses such as integrin activation and the release of ADP and thromboxane A2 (TxA2) that can activate the G protein-coupled receptors (GPCRs), ADP purinergic receptors P2Y1 (7) and P2Y12 (8) and the thromboxane receptors (TP) TP α and TP β . These second wave mediators allow the recruitment of circulating platelets which reinforce thrombus formation on collagen surfaces (9). In the clinic, pharmacological inhibition of TxA2 generation and/or the P2Y12 receptor are effective strategies to reduce thrombus formation at sites of vascular injury (10). Importantly, GPVI plays a central role in collagen-induced exposure of procoagulant phospholipids at the platelet surface, allowing efficient thrombin generation (11) and platelet activation. Of note, human platelets express the thrombin receptors, PAR1 and PAR4, whereas mouse platelets express a PAR3/PAR4 complex (12).

The specificity of GPVI is not restricted to collagen. Laminin as well as fibronectin, present in the basement membrane, have been shown to support platelet adhesion and spreading through $\alpha 6\beta 1$ and GPVI (13, 14). The exclusive expression of GPVI on platelets makes it an attractive target. A recent placebo-controlled phase 1 study evaluated the safety and tolerability of a humanized Fab anti GPVI (Act017) in healthy donors. This study reported no bleeding events or increased of the bleeding time suggesting a promising effect of targeting GPVI in thrombotic diseases (15). The hemITAM receptor, C-type lectin 2 (CLEC2), may also contribute to platelet activation and

thrombus formation in the deeper layers of the ECM. The known ligand for CLEC-2 is podoplanin which is expressed by type-1 alveolar cells, fibrotic reticular cells, lymphatic endothelial cells (EC) but not by vascular ECs. While the role of CLEC-2 in arterial thrombosis is still not clear (16–18), a recent *in vitro* study shows that podoplanin-expressing perivascular mesenchymal stromal cells are able to protrude through ECs and activate platelets in a CLEC-2 dependent manner (19). However, further *in vivo* studies are needed to support this observation in the context of inflammation or vascular injury.

Platelets are also known to play an important role in inflammation by recruiting leukocytes. This crosstalk has been well-studied and contributes to the increased leukocyte infiltration in tissue. The molecular mechanism of this crosstalk has been experimentally documented in different organs and in various inflammatory situations. Experimental studies show that thrombocytopenic animals have a significant reduction in leukocyte numbers in inflamed organs (20–22). Similarly, mice deficient for the main platelet adhesion receptors (P-selectin, GPIb α , GPVI, $\beta 3$ integrin) show reduced tissue inflammation (23–25). Importantly, the direct interaction between platelets and leukocytes not only occurs locally but also in circulation. Increased levels of neutrophil/platelet and/or monocyte/platelet aggregates have been reported in patients with various inflammatory diseases (26–28). Platelet activation and secretion have been shown to recruit leukocytes, upregulate adhesion molecules by EC and destabilize EC junctions. Platelets can release a variety of chemokines (e.g., platelet factor 4, IL1 β , PAF, RANTES) that can up regulate endothelial adhesion molecules (ICAM, $\alpha v\beta 3$) (29), as well as the release of Weibel palade content and open endothelial junctions (30). Among the pro-permeable platelet factors, the GPVI-dependent serotonin release has been shown to contribute to the inflammation in the joints of arthritic mice (31). Furthermore, the release of soluble factors by platelets is also central to stimulate leukocytes. For example, platelet-released adenine nucleotides or platelet factor 4 and PDGF can favor superoxide anion generation by neutrophils (32). Conversely, activated neutrophils through leukocyte-released substances, such as platelet-activating factor (PAF), elastase, and cathepsin G, may induce platelet aggregation and secretion (33). The overall effect of the platelet-leukocytes interaction on tissue integrity can be either beneficial or deleterious depending of the inflammatory situation. This dichotomous aspect of the platelet-leukocyte interaction is further documented by the formation of neutrophil extracellular traps (NETs) that entrap bacteria (34) but also cause significant damage to the surrounding tissue (35). Based on the intertwined interaction between platelet and leukocyte in pathological situations, platelet/leukocyte aggregates (PLA) are rather important regulators of disease than just a read-out for platelet activation. The use of platelet inhibitors in patients with cardiovascular disease (e.g., clopidogrel, aspirin, eptifibatide) has been shown to reduce leukocyte recruitment, cytokine release and subsequently improve disease outcome. For instance, clopidogrel pretreatment in addition of ASA therapy was shown to reduce the C-Reactive protein (CRP) level, an inflammatory marker, in patients with percutaneous

Abbreviations: AAA, abdominal aortic aneurysm; ACS, acute coronary syndrome; BAPN, beta 3-aminopropionitrile fumarate salt; CalDAG-GEFI, calcium and diacylglycerol-regulated guanine nucleotide exchange factor I; CLEC2, C-type lectin 2; COX-1, cyclooxygenase-1; CVD, cardiovascular diseases; DAG, diacylglycerol; DAPT, dual antiplatelet treatment; EC, endothelial cells; ECM, extracellular matrix; FcR γ , Fc receptor γ chain; GPCRs, G protein-coupled receptors; GPIb α , glycoprotein Ib α ; GPVI, glycoprotein VI; ILT, intra-luminal thrombus; IR, ischemia-reperfusion; ITAM, immunoreceptor tyrosine-based activation motif receptors; MI, myocardial infarction; MMP, matrix-degrading proteinases; NETs, neutrophil extracellular traps; oxLDL, oxidized low density lipoproteins; PAF, platelet-activating factor; PAR, protease-activated receptor; PDGF, platelet derived growth factor; Pg, *Porphyromonas gingivalis*; PKC, protein kinase C; PLA, platelet-leukocyte aggregates; PLD1, phospholipase D1; rt-PA, recombinant tissue plasminogen activator; ROS, reactive oxygen species; STEMI, ST-segment elevation myocardial infarction; tMCAO, transient middle cerebral artery occlusion model; TP, TxA2 receptor; TxA2, thromboxane A2; vWF, von Willebrand factor.

coronary intervention (36). Apart from antiplatelet therapy, anti-inflammatory agent such as colchicine has been used in various settings of cardiovascular disease (e.g., myocardial infarction) by inhibiting interleukin-1 production by neutrophils (37, 38). Considering the anti-inflammatory properties of antiplatelet therapy, one could speculate that antiplatelet therapy could be considered as either a complementary or a second line of treatment to inflammatory medications (e.g., colchicine) in vascular disease.

ROLE OF PLATELETS IN EARLY AND LATE STAGES OF ATHEROSCLEROSIS

Atherosclerosis is a chronic inflammatory vascular disease involving ECs, vascular smooth muscle cells and mononuclear cells. Atherosclerosis classification as an inflammatory disease is based on the finding that immune competent cells and pro-inflammatory cytokines are abundant in atherosclerotic lesions. It is characterized by the formation of an atheromatous plaque mainly composed of pro-inflammatory oxidized low density lipoproteins (oxLDL) and foam cells accumulation in the intima of medium or large arteries, in high-shear stress areas. It results in vessel occlusion inducing CVD onset (39). The role of platelets in early atherosclerosis have been proposed by pioneer studies. Russel Ross postulated in his “*response to injury theory*” that “*lesions of atherosclerosis result from injury to the artery wall*” and result in “*subtle arterial endothelial cells desquamation*” (40). Of the many possible injuries, mechanical stresses may occur at particular anatomic sites and lead to the detachment of ECs from the artery wall and subsequently platelet adherence (41). Recent studies also reported endothelial breaches in the intima of human coronary arteries as well as in ApoE mice at sites of flow perturbation leading to the infiltration of red blood cells and leukocytes (42). Almost 30 years after Russel Ross hypothesis, the Massberg group in a landmark paper showed that in high-fat diet fed ApoE mice, platelet adhesion to the endothelium precedes the development of atherosclerotic lesions and leukocyte recruitment in atherosclerotic plaque supporting a major role of platelets in atherogenesis (43). Mechanistic studies showed that prolonged blockade of platelet adhesion in atherosclerosis animal model reduces leukocyte recruitment in arterial wall and results in fewer lesion formation (43). To decipher the involvement of platelets in this disease, several genetically modified mice lacking diverse platelet receptors were used in animal models of atherosclerosis.

Platelet glycoprotein Ib α (GPIb α), the ligand-binding subunit of the GPIb-V-IX receptor complex is known to interact with several proteins like vWF, P-selectin, Mac-1 and α -thrombin (44–47). Injections of anti-GPIb α antibodies in 10 weeks old ApoE $^{-/-}$ mice reduced both platelet transient and firm adhesion to the vascular surface of the common carotid. Genetic depletion of the GPIb α subunit leads to severe thrombocytopenia and reduced atherosclerosis progression with smaller lesion area (48). This reported protective effect could be a consequence of thrombocytopenia since mice with extracytoplasmic GPIb α domain genetic deletion (IL4R/GPIb α mice) develop milder thrombocytopenia and are not protected against atherosclerosis

(48). It indicates that GPIb α binding site for vWF, P-selectin, Mac-1 and α -thrombin might be dispensable for atherosclerosis development. This is quite surprising since vWF genetic depletion is protective in an animal model of atherosclerosis (49). Similarly, a role for Mac1/GPIb α interaction has been shown in leukocyte recruitment at sites of vascular inflammation (50, 51). The subunit GPIIb β has been also investigated in atherosclerosis by using GPIIb $\beta^{-/-}$ /ApoE $^{-/-}$ mice fed a chow diet for 30 weeks. Despite the moderate thrombocytopenia of those mice, GPIIb β was found dispensable in atheroprotection (52).

Overall, these studies suggest that redundant mechanisms in platelet recruitment occur at site of developing atherosclerosis. The integrin α IIb β 3 can also mediate platelet adhesion via vWF binding, especially in modest shear stress condition as in large arteries. GPIIb genetic depletion results in reduced platelet adhesion at sites of vascular injury, decreased inflammatory processes and fewer atherosclerotic plaque formation (53). Integrin α IIb β 3 activation can be induced by GPIb-V-IX receptor, but also by GPVI receptor (54). Several GPVI inhibition strategies led to reduced platelet adhesion and attenuated atherosclerosis in ApoE $^{-/-}$ mice (55).

Aside from platelet adhesion, platelet activation plays a significant role in atherogenesis. The presence of activated platelets was reported in the blood obtained from patients with unstable atherosclerotic disease (56). Increased platelet reactivity has been suggested as a potential mechanism contributing to the accelerated atherosclerosis seen in diabetic patients, via capillary microembolization and acute arterial thrombosis (57). Likewise, circulating activated platelets are involved in the formation of atherosclerotic lesions in ApoE $^{-/-}$ mice (58). Those activated platelets interact with the atherosclerotic endothelium, leading to the delivery of pro-inflammatory chemokines (e.g., CCL5 and CXCL4) promoting adhesion molecule expression (58). PLA formation is required for neutrophil recruitment to inflamed tissues as animal models studies revealed that platelets activate neutrophils for an efficient adhesion to vascular endothelium via integrin up-regulation (59). Platelets can also bind to the inflamed endothelium, enhancing leukocytes adhesion to the vessel wall (60–62). Indeed, deletion of P-selectin, a marker of granule secretion, in platelets and/or ECs leads to significantly impaired early atherosclerotic lesion development in mice (63, 64). In addition, several studies addressing the contribution of platelet receptors have been conducted. The role of the ADP receptor P2Y₁₂ has been extensively studied throughout the years. It has been shown that P2Y₁₂ genetic depletion is protective in ApoE $^{-/-}$ mice and mediates a reduced lesion area, an increased fibrous content at the plaque site and less inflammatory cells infiltration (65). However, the role of P2Y₁₂ expressed by vascular smooth muscle cells cannot be excluded (66). Mice deficient for P2Y₁₂ specifically in hematopoietic cells were generated, and a reduced atherosclerotic lesion formation was also reported (67). In contrast, treatment of ApoE $^{-/-}$ mice with the P2Y₁₂ inhibitor clopidogrel bisulfate was associated with inconsistent results. Clopidogrel administration induced delayed atherogenesis, a reduced lesion size, slower progression of atherosclerotic lesion (68–70). However, another study reported that clopidogrel-treated mice

have the same atherosclerotic burden as control mice (70). Moreover, clopidogrel administration in mice with established atherosclerotic lesions show no longer beneficial effect (69). A more recent P2Y₁₂ inhibitor, ticagrelor, has been tested in atherosclerosis models. Several studies conclude to a beneficial effect of ticagrelor administration, reporting a reduced lesion area and slower atherosclerotic lesion progression (71, 72). Nevertheless, one study related no effect on atherosclerotic lesion size in ticagrelor-treated mice, but showed an increased fibrous cap area along with a diminished ratio necrotic core/lesion area, indicating plaque stabilization process (73). Pharmacological inhibition is more likely to give variable results than a genetic approach. The differences in the experimental conditions and the inhibitor dose may be responsible for the discrepant results. The impact of the platelet thrombin receptor, PAR4, has been also investigated in atherosclerosis. Indeed, transfusion of thrombin-activated platelets into mice increases plaque formation, suggesting that thrombin-induced platelet activation might contribute to platelet-dependent atherosclerosis (58). However, PAR4 deletion is not protective in ApoE^{-/-} mice (74) suggesting other platelet activators than thrombin are involved.

Upon activation, platelets release soluble factors (e.g., PF4, CD40L, RANTES, and TXA₂) enhancing their activation and leukocyte recruitment. Disruption of this amplification process leads to diminished atherosclerotic lesion formation. Indeed, PF4 or CD40L genetic deletion protects ApoE^{-/-} mice from atherosclerosis (75, 76). Inhibition of RANTES or its receptors alters the progression of an established atherosclerotic lesion (77, 78). Biological response modulators such as CD40L and its receptor CD40 have been shown to exacerbate atherosclerosis progression by promoting leucocyte recruitment via molecule adhesion expression in vascular ECs (79).

Platelet TXA₂ generation is the product of cyclooxygenase-1 (COX-1) activation and contributes to the platelet activation amplification loop. The TXA₂ receptor (TP) antagonist administration induces a slight reduction of atherogenesis (80), and TP deficient ApoE^{-/-} mice showed delayed lesion development and reduced atherogenesis compared to control (81). Disruption of COX-1 expression in ApoE^{-/-} mice induces a decrease in atherosclerotic lesion formation, attesting TXA₂ deleterious role in this pathology (82). Acetylsalicylic acid, also known as aspirin, is one of the most widespread antiplatelet treatment and displays also anti-inflammatory properties. This irreversible COX-1 inhibitor blocks the formation of TXA₂ in platelet, producing an inhibitory effect on platelet aggregation. Most animal studies reported a beneficial effect of low-dose aspirin administration in ApoE^{-/-} mice. Atherogenesis and lesion progression is reduced in aspirin-treated mice compared to control (83–85). Low-dose aspirin also delayed the progression of established and advanced vascular atherosclerotic lesions (86). However, some studies reported no effect of aspirin in ApoE^{-/-} mice (70, 80), and one described a deleterious long-term effect on atherosclerotic lesion progression (87). Overall, these studies suggest a functional hierarchy and redundancy between the different receptors in the role of platelets in atheroprotection.

Downstream of the platelet receptors, the signaling molecule GTPase Rap1 is a critical node in platelet response. The calcium and diacylglycerol-regulated guanine nucleotide exchange factor I (CalDAG-GEFI; RasGRP2) has been identified as the major calcium sensor in platelets regulating the Rap1 activation (88). Studies led by the Bergmeier group uncovered key roles of CalDAG-GEFI in platelet responses: integrin activation, platelet adhesion and secretion, TXA₂ generation (89, 90). In an animal model of atherosclerosis, mice lacking CalDAG-GEFI specifically in hematopoietic cells have smaller lesions, reduced atherogenesis and decreased inflammation in areas of plaque development compared to control mice (67).

Even though the stenosis induced by atherosclerosis can restrict blood flow and thus induces CVD, the main mechanism implied in those diseases seems to be atherothrombosis. Indeed, following plaque rupture, prothrombotic materials (collagen and tissue factor) are exposed to the blood coagulation system leading to thrombus formation, decreased blood flow and CVD onset (39). Human postmortem studies showed that thrombi that form on disrupted plaques (e.g., asymptomatic coronary disease) appear small and non-occlusive (91). Animal models that can recapitulate spontaneous rupture of atherosclerotic lesions are very rare. To circumvent this issue, two experimental animal models have been developed to study platelet mechanism in thrombosis-induced plaque rupture. A model of ultrasound-induced plaque injury and a model of acute plaque rupture using a suture needle have been developed to test antiplatelet drugs in mice (92, 93). Ultrasound treatment resulted in a fissure at the shoulder region of the plaque leading to plaque material exposure (collagen) and unstable thrombus formation. Unlike the ultrasound model, the needle model is characterized by a frank rupture with larger and stable thrombi. At the site of plaque rupture, smaller thrombi were observed after P2Y₁₂ or thrombin or integrin α Ib β 3 inhibition in both models (92–94). The role of GPVI seems to be more pronounced in the ultrasound model presumably due to a higher amount of thrombin generated in the needle model (93). The role of the different platelet molecules in mouse atheroprotection has been summarized in **Table 1**.

The protective role of these drugs is difficult to assess in human clinical trials since atherosclerosis by itself is almost always asymptomatic. Thereby, their efficacy is studied in CVD with an atherosclerotic origin such as myocardial infarction (MI) and stroke.

PLATELETS CONTRIBUTE TO THROMBO-INFLAMMATION DURING STROKE

According to the World Health Organization, an estimated 7 million people died from stroke worldwide in 2016. Stroke represents the second most common cause of death and the third most common cause of disability (95). Strokes have mainly an ischemic origin (70%) and occur when an artery that supplies blood to the brain is blocked by a blood clot (96). Hemorrhagic stroke accounts for 15% of all strokes but they are responsible for about 40% of all stroke deaths (National Stroke Association).

TABLE 1 | A comprehensive analysis of platelet mechanisms in atheroprotection in mice (↓ decrease, ↑ increase, = no effect on plaque development).

Molecule targeted	Animal model	Plaque development effect	References
PGI ₂	ApoE ^{-/-} IP ^{-/-} (chow diet)	↑	(81)
GPIIb α	ApoE ^{-/-} + Fab anti-GPIIb α (HFD up to 18 weeks)	↓	(43)
	Ldlr ^{-/-} GPIIb α ^{-/-} (HFD for 16 weeks)	↓	(48)
	Ldlr ^{-/-} hIL4R/GPIIb α (HFD for 16 weeks)	=	(48)
vWF	Ldlr ^{-/-} vWF ^{-/-} (HFD up to 22 weeks)	↓	(49)
α IIb β 3	ApoE ^{-/-} GPIIb ^{-/-} (HFD for 8 at 12 weeks)	↓	(53)
GPVI	ApoE ^{-/-} + Fab anti-GPVI (HFD for 12 weeks)	↓	(55)
PAR4	ApoE ^{-/-} PAR4 ^{-/-} (HFD for 5 or 10 weeks)	=	(74)
CD40L	ApoE ^{-/-} CD154 ^{-/-} (chow diet)	↓	(75)
CD40	ApoE ^{-/-} CD40 ^{-/-} (HFD for 4 weeks)	↓	(79)
PF4	ApoE ^{-/-} PF4 ^{-/-} (HFD for 10 weeks)	↓	(76)
RANTES	Ldlr ^{-/-} + RANTES inhibitor (HFD up to 22 weeks)	↓	(77, 78)
TXA ₂	ApoE ^{-/-} + S18886 or aspirin (chow diet)	↓	(80)
	ApoE ^{-/-} COX-1 ^{-/-} (HFD for 8 weeks)	↓	(82)
	Ldlr ^{-/-} + aspirin (chow diet or HFD up to 26 weeks)	↓	(83–85)
	ApoE ^{-/-} + aspirin (chow diet or HFD up to 12 weeks)	=	(70, 80)
	ApoE ^{-/-} + aspirin long treatment (HFD up to 16 weeks)	↑	(87)
CalDAG-GEFI	CalDAG-GEFI ^{-/-} in hematopoietic cells (HFD for 12 weeks)	↓	(67)
P2Y ₁₂	ApoE ^{-/-} P2Y ₁₂ ^{-/-} (HFD up to 20 weeks)	↓	(65, 67)
	ApoE ^{-/-} + Clopidogrel (chow diet or HFD between 8 at 12 weeks)	↓	(68, 70)
	ApoE ^{-/-} + Clopidogrel (HFD for 6 months)	=	(69)
	ApoE ^{-/-} + Ticagrelor (HFD for 20 weeks)	↓	(71, 72)
	ApoE ^{-/-} + Ticagrelor (HFD for 12 weeks)	=	(73)

An ischemic stroke can occur in two ways: embolic stroke caused by thromboembolism of cardiac origin or thrombotic stroke with *in-situ* blood clot. Atherosclerosis in major intracranial arteries leads to changes ranging from minor wall thickening to luminal stenosis, and is one of the most common causes of

stroke worldwide (97). The middle cerebral arteries are the most common lesion site, followed by the basilar artery, the internal carotid arteries, and the intracranial vertebral arteries (98). Intracranial atherosclerotic disease may occur concomitantly with systemic atherosclerosis.

The current treatments for acute ischemic stroke are the use of a thrombolytic agent as recombinant tissue plasminogen activator (t-PA) (99) and mechanical thrombectomy (100). Nevertheless, in some patients, the recanalization by thrombolysis is not efficient and the persisting thrombus leads to severe brain damage. Recent clinical findings show that clots retrieved from stroke patients have a thick compact outer shell enriched in NETs and fibrin which might contribute to reperfusion resistance (101, 102). Following thrombolysis, the overall recanalization rate is 46% (103). However, reocclusion after initial recanalization occurs in 14–34% of patients and is associated with clinical deterioration and poor outcome (104–106). Reocclusion has been attributed to increased platelet aggregation caused by the local thrombus and endothelial injury. Thus, the start of antiplatelet therapy early after thrombolysis might reduce the risk of reocclusion and thereby improve functional outcome.

Animal models were used to decipher platelets involvement in stroke. The most common stroke animal model used is the transient middle cerebral artery occlusion (tMCAO) in mice and rats. Thrombocytopenic mice were submitted to transient occlusion of the middle cerebral artery, and 24 h after ischemia/reperfusion, infarct area was determined (107). Platelet depletion did not significantly affect the lesion area, but thrombocytopenic mice presented multiple hemorrhagic foci in the lesion whereas none were observed in mice with normal platelet count. Nevertheless, platelet adhesion and activation have been investigated in several stroke studies in mice. Blockade or genetic deficiency of GPIIb α improves stroke outcome without hemorrhagic transformation after tMCAO (108). Similarly, vWF deficiency is also associated with smaller infarct volumes and no bleeding was observed after tMCAO in those mice (109). Interestingly, the role of the GPIIb α -vWF axis in hemostasis can be decoupled from the one in brain injury highlighting a proinflammatory role of GPIIb α . The contribution of platelet activation receptors has been investigated during ischemia-reperfusion injury after tMCAO. Inhibition or genetic deficiency of GPVI, the collagen and fibrin receptor, has been shown to reduce the infarct volume and to improve stroke outcome (108, 110). Supporting the beneficial role of blocking the collagen receptor during a stroke, a recent study showed that GPVI inhibition plus intravenous infusion of rt-PA is safe in term of bleeding and has a better outcome than rt-PA alone (111). GPVI seems an attractive target in stroke since (i) it's only expressed on platelets, (ii) patients with a GPVI deficiency have no or mild bleeding phenotype (112), (iii) GPVI inhibition leads to a significant reduction of thrombus formation (113, 114), (iv) a novel humanized Fab anti GPVI (ACT017) in healthy donors didn't show bleeding complications (15). Overall, targeting GPVI in thrombosis can be a novel approach and compared to the current antiplatelet drugs, GPVI inhibition does not compromise hemostasis. Nevertheless, other larger studies

need to be conducted in patients with CVD to validate the use of anti-GPVI antibodies. Notably, the protective effect of GPIIb α and GPVI inhibition can also be observed in aged mice presenting comorbid factors such as atherosclerosis, diabetes or hypertension (115). Indeed, platelets from patients with comorbid factors are in a hyperactivated state (116). Enhanced platelet intracellular calcium responses to LDL cholesterol have been observed in diabetic patients with and without hypertension (117). Similarly, platelets from diabetic patients have been reported to have reduced sensitivity to prostacyclin (118) and hyperaggregate in response to platelet agonists (119). Other changes in platelets from diabetic patients include an increased expression of some platelet receptors GPIIb α and α IIb β IIIa (120) and an alteration of platelet membrane fluidity (121). Therefore, studies including comorbid factors should be performed to further assess the validity of future antiplatelet drugs in the context of CVD.

Other platelet receptors are involved in platelet activation during the ischemia-reperfusion mouse model. The thrombin PAR4 receptor is expressed not only in platelets but also in the central nervous system (122). In a transient stroke mouse model, its systemic deletion reduces the brain infarct volume and attenuates cognitive function deficit (123). Intravital microscopy studies showed fewer platelet/EC interactions in PAR4^{-/-} mice compared to control mice. Thus, PAR4 deficiency seems to be neuroprotective in transient middle cerebral artery occlusion, partially through the attenuation of cerebral microvascular inflammation. In addition, mice deficient for G α ₁₂, G protein downstream of the ADP receptor P2Y₁₂, were subjected to tMCAO, then functional outcome and infarct size were assessed 24 h later (124). G α ₁₂ deficiency leads to a reduced lesion area and better functional outcome than control mice. Apart from platelet adhesion and activation, platelet granule secretion contributes to stroke. Mice lacking platelet α -granules (Nbeal2^{-/-}) and mice lacking platelet dense granules (Unc13d^{-/-}) showed a higher mortality rate due to intracranial hemorrhage. Nevertheless, the surviving animals developed significantly smaller brain infarctions and had a better outcome compared to WT mice (125). Platelet aggregation mediated by the integrin α IIb β 3 seems dispensable at sites of ischemia/reperfusion injury. Blockade of α IIb β 3 integrin increases intracranial hemorrhage risk in tMCAO mice (108, 126) and among the surviving mice, the treatment did not show any improvement (115) suggesting that platelet aggregation is dispensable for brain injury but still important for securing hemostasis in tMCAO.

Overall, these data suggest a deleterious role of platelet adhesion, activation and secretion in the stroke pathophysiology. On the opposite, platelet aggregation, which plays a crucial role in thrombus formation, is not required for stroke progression. Thus, the non-classical role of platelets, through their pro-inflammatory properties, may prevail in stroke disease. A comprehensive table summarizes platelet mechanisms studied in tMCAO models (**Table 2A** and **Figure 1**).

In humans, several antiplatelet drugs have been tested in stroke outcome. Among them, numerous studies have investigated the benefits and risks of aspirin for primary prevention in population at risk, during the acute management of cardiovascular events and in secondary prevention among

patients with CVD. In elderly populations, the risk of CVD is higher suggesting an increased benefit of aspirin administration in primary prevention for cardiovascular events. However, increased bleeding risk has also been observed in this population (147). According to a meta-analysis of aspirin primary prevention studies, reported by the Antithrombotic Trialists' Collaboration, aspirin did not reduce the risk of stroke. In this meta-analysis, aspirin non-significantly reduced the risk of ischemic stroke but increased non-significantly the risk of hemorrhagic stroke (148). The JPPP (Japanese Primary Prevention Project) clinical trial was designed to assess whether primary prevention would reduce the risk of non-fatal stroke in elderly Japanese patients (127). It appears that aspirin seems to reduce the non-fatal ischemic stroke risk, but it tends to increase the risk of hemorrhagic stroke. Recently, the ARRIVE (Aspirin to Reduce Risk of Initial Vascular Events) clinical trial investigated the benefits and risks of enteric-coated aspirin used in primary prevention of cardiovascular events, in patients with an average cardiovascular risk (128). Aspirin did not lower the risk of major cardiovascular events nor stroke incidence. Moreover, rates of gastrointestinal bleeding events and some other minor bleeding events were higher in the aspirin treatment group with no difference in the incidence of fatal events. The results of the ASPREE (Aspirin in Reducing Events in the Elderly) clinical trial investigating the role of aspirin in primary prevention of CVD were published recently (129). They confirm that the use of low-dose aspirin as a primary prevention strategy in older adults results in a significantly higher risk of major hemorrhage and did not trigger a significantly lower risk of CVD than placebo.

Low-dose aspirin efficacy has been widely established in secondary prevention trials, in which the benefits of reducing ischemic stroke rates have outweighed the risk of hemorrhage (149, 150). A meta-analysis including eight clinical trials involving more than 40,000 participants concluded that antiplatelet therapy with aspirin started within 48 h of the onset of ischemic stroke reduced the risk of early recurrent ischemic stroke without a major risk of early hemorrhagic complications (151). Moreover, long-term outcomes were also improved. The ARTIS (Antiplatelet therapy in combination with rt-PA Thrombolysis in Ischemic Stroke) clinical trial compared the effects of early intravenous aspirin addition to thrombolysis with standard treatment without aspirin (130). Patients with acute ischemic stroke treated with rt-PA thrombolysis were randomly assigned to intravenous aspirin within 90 min after the start of thrombolysis treatment or to no additional treatment. In addition, in both groups, oral antiplatelet therapy was started 24 h after thrombolysis treatment. This trial concluded that early administration of intravenous aspirin does not improve outcome at 3 months and increases the risk of intracranial hemorrhage without evidence of a beneficial effect on early neurological deterioration (131).

Current guidelines for the early management of patients with acute ischemic stroke, from the American Heart Association and the American Stroke Association, recommend starting aspirin administration 24 h after thrombolysis (152). However, the overall effect of aspirin in acute ischemic stroke is weak and better acute therapies are therefore necessary.

TABLE 2 | (A) A comprehensive analysis of platelet mechanisms in tMCAO mouse model and **(B)** in stroke patients (↓ decrease, ↑ increase, = no effect on infarct area or bleeding risk).

A				
Molecule targeted	Animal model	Effect on infarct area		References
Platelets	Thrombocytopenia	=		(107)
α granules	Nbeal2 ^{-/-}	↓		(125)
Dense granules	Unc13d ^{-/-}	↓		(125)
PAR4	PAR4 ^{-/-}	↓		(123)
Gα _{i2}	Gαi2 ^{fl/fl} /PF4-Cre	↓		(124)
GPIIbα	Antibody anti-GPIIbα	↓		(108, 126)
GPVI	Antibody anti-GPVI	↓		(108)
αIIbβ3	Antibody anti-GPIIb/IIIa	↓		(108)
B				
Molecule targeted	Clinical trial	Effect	Bleeding	References
TXA ₂	JPPP	↓	↑	(127)
	Aspirin			
	ARRIVE	=	↑	(128)
	Aspirin			
	ASPREE	=	↑	(129)
	Aspirin			
P2Y ₁₂	ARTIS	=	↑	(130, 131)
	Early aspirin addition to thrombolysis			
	CAPRIE	=	↑	(132)
	Clopidogrel vs. aspirin - Ischemic stroke patients			
	CHARISMA	=	↑	(133)
	Clopidogrel + aspirin vs. aspirin alone			
	MATCH	=	↑	(134)
	Clopidogrel + aspirin vs. aspirin alone			
	CHANCE	↓	=	(135, 136)
	Clopidogrel + aspirin vs. aspirin alone			
	COMPRESS	=	↑ Tendency	(137)
	Clopidogrel + aspirin vs. aspirin alone			
	POINT	↓	↑	(138, 139)
	Clopidogrel + aspirin vs. aspirin alone			
SOCRATES	=	=	(140, 141)	
Ticagrelor vs. aspirin				
PRINCE	↓ Tendency	?	(142, 143)	
Ticagrelor vs. clopidogrel (+ aspirin)				
TARDIS	=	↑	(144)	
Aspirin, clopidogrel and dipyridamole vs. guideline treatment				
αIIbβ3	abESTT-II	=	↑	(145)
	Abciximab			
	SaTIS	=	=	(146)
	Tirofiban vs. placebo			

Over the years, several other antiplatelet agents were developed and then tested in clinical trials. A comprehensive table summarizes the different clinical trials that assessed the efficacy of antiplatelet agents in stroke outcome (**Table 2B**).

PLATELETS CONTRIBUTE TO MYOCARDIAL INJURY

Acute coronary syndrome (ACS) occurs when the blood flow is decreased or stopped in coronary arteries, leading to tissue

damage ranging from ischemia to infarction. This defect of blood supply is mainly due to atherosclerotic plaque growth and rupture, followed by a thrombus formation in coronary artery (153). ACS is commonly divided into myocardial infarction (MI) and unstable angina, considered to be an imminent precursor of MI. Biomarkers of cardiac tissue damage such as troponin and creatine-kinase myocardial band (CK-MB) are used to classify ACS into unstable angina or MI, the latter one presenting such blood biomarkers. A MI can have several consequences such as heart failure, an irregular heartbeat or a cardiac arrest.

According to the World Health Organization, in 2012 an estimated 7.5 million people died from MI worldwide. Survivors of MI are at increased risk of recurrent infarctions and have an annual death rate of 5%, representing a 6-time increase compared to people without coronary heart disease. MI can also be classified into ST-segment elevation myocardial infarction (STEMI) and non-STEMI (NSTEMI) according to the patient electrocardiogram. STEMI caused by a complete coronary vessel occlusion, and NSTEMI due to a partial artery occlusion, represent respectively 30 and 70% of all MI (154). Since unstable angina has similar pathophysiology to NSTEMI, they are referred together as non-ST-segment elevation ACS (NSTEMI-ACS) and are grouped for care management decisions. Current guidelines recommend an immediate treatment of ACS, but due to different pathophysiology between STEMI and NSTEMI-ACS, separate guidelines were edited. Guidelines for the management of NSTEMI-ACS recommend a pharmacological treatment of ischemia (via decreasing myocardial oxygen demand or increasing myocardial oxygen supply) (155). Guidelines for the treatment of STEMI recommend an immediate recanalization of coronary arteries via reperfusion therapies such as primary percutaneous coronary intervention and/or fibrinolysis strategy (156). Even though recanalization is necessary to provide oxygen and nutrients to the ischemic area, reperfusion by itself also exacerbates myocardial damage (157). This pathologic process is named ischemia-reperfusion (IR) injury. For long-term therapies, both guidelines agree to strongly recommend the use of antiplatelet agents.

Indeed, the atherosclerotic origin of MI led to numerous studies deciphering the platelet involvement in MI. A lower platelet count or no significant difference of the platelet count was observed between MI patients and stable angina or healthy donors (158, 159). However, the mean platelet volume reflecting platelet activation was higher in MI patients compared to stable coronary artery disease patients at the time of acute event (158). Increased levels of P-Selectin and CD63-exposing platelet microparticles have been found in MI patients (160). Plasma levels of vWF and serotonin are increased in patients with coronary artery syndrome (161, 162) highlighting the role of platelet activation in myocardial injury. Moreover, platelet-leucocyte aggregates are an early marker of acute MI and are also associated with myocardial no-reflow in STEMI patients (163, 164). Ventricular wall rupture is a fatal complication of acute MI and platelets seem to be involved in this phenomenon since an intramural thrombus was observed within the infarcted myocardium (165). Platelets potential involvement in this process was confirmed by their depletion which reduced the rate of myocardial wall rupture from 46 to 0% (166). Several studies investigated platelet mechanisms involved in MI and in myocardial (IR) injury.

Platelet activation is commonly observed in numerous pathologies, including MI (158). This phenomenon is mediated by specific platelet receptors that are involved in adhesion and activation. Inhibition of GPIIb/IIIa—involved in platelet tethering—via a Fab anti-GPIIb/IIIa did not change the infarct volume per the area at risk (INF/AAR) (167). In accordance with the results of GPIIb/IIIa inhibition, depletion of the phospholipase D1 (PLD1), enzyme involved in GPIIb/IIIa dependent α IIB β 3 activation,

did not protect mice from myocardial IR injury (167). These findings suggest that GPIIb/IIIa platelet receptor is dispensable in MI pathophysiology. On the contrary, mice deficient for the Fc receptor γ chain (FcR γ) coupled to GPVI were protected from myocardial IR injury with smaller infarct size and reduced leucocyte recruitment in the injured area (168). This was confirmed by pharmacological inhibition of the collagen receptor GPVI via a Fab anti-GPVI or soluble GPVI-Fc (Revacept) in a mouse model of the left anterior descending artery ligation with reperfusion. Inhibition of GPVI led to a reduced infarct size (167, 169). These findings suggest that a therapeutic strategy targeting GPVI could be a valuable approach in MI. This could be relevant in humans since it was recently described that patients with STEMI have an alteration of GPVI platelet signaling (170). Indeed, platelets from STEMI patients have an increased aggregation response compared to stable coronary artery disease patients. This could be due to the increased number of GPVI receptors observed in ACS patients (171). Therefore, studies focused on GPVI inhibition seem promising. Currently, a humanized Fab targeting GPVI without increasing the bleeding risk in healthy controls is developed and characterized (15) but their findings warrant further investigations in CVD patients under current antiplatelet drugs.

The contribution of other receptors in MI was studied such as CLEC-2, PAR receptors or P2Y₁₂ in mice. Pharmacological inhibition of CLEC-2 via Fab administration did not decrease the INF/AAR in mice (167). The thrombin receptors, PAR1 and PAR4 receptors are expressed at the surface of platelets, but also by cardiomyocytes (172, 173). It has been showed that PAR1 deficiency did not affect the infarct size after myocardial IR injury (174). However, interestingly, PAR1^{-/-} mice had reduced cardiac remodeling and decreased impairment of left ventricle function compared to control mice. In contrast to previous findings, PAR1 antagonist (SCH 79797) administration was shown to reduce infarct size after myocardial IR injury in rats (175). This discrepancy could be explained by off-target effects of SCH 79797 or a PAR4 compensation. Genetic depletion of PAR4 led to the development of larger infarcts and more myocardial apoptosis compared to control mice (176). However, another study attributes a cardioprotective effect of PAR4 deletion after myocardial IR injury as INF/AAR was decreased in PAR4^{-/-} mice compared to control mice (177). The administration of PAR4 antagonists confirmed the previous findings. Indeed, PAR4 inhibition in rats decreased infarct size after myocardial IR injury (178). The P2Y₁₂ receptor, which signaling is mediated by the G protein G α_{i2} , is involved in platelet activation. G α_{i2} ^{-/-} deficient mice have a reduced INF/AAR ratio suggesting a protective effect of platelet activation inhibition (124). However, this G protein may interact not only with P2Y₁₂, but also with additional G protein coupled receptor present in platelets. The use of clopidogrel, a P2Y₁₂ antagonist, decreased platelet accumulation in ischemic myocardium and reduced the rupture rate from 45% in control to 10% in clopidogrel treated animals (165, 166). In a rat model of isolated hearts, the perfusion of platelets from acute MI patients enlarges infarct area (179) while the concomitant administration of cangrelor or abciximab decreases the infarct size. As opposed to clopidogrel, aspirin administration did not

reduce infarct size nor the rupture rate in mice (165, 180) suggesting a relative hierarchy in the platelet receptors during MI.

Platelet degranulation, a marker of platelet activation, triggers the inflammatory responses by P-selectin exposure involved in leukocyte recruitment. Nbeal2^{-/-} and Unc13d^{-/-} mice lacking, respectively, alpha and dense granules did not show any alterations in infarct sizes, arguing against a significant role of degranulation in the pathophysiology of myocardial IR injury (167). Nevertheless, blockade or genetic deficiency of P-selectin can lead to smaller infarct sizes after myocardial infarction (181–183) suggesting a possible contribution of the endothelial P-selectin. Critical to platelet activation is calcium mobilization. Cytosolic Ca²⁺ concentration is regulated by two major proteins: STIM1 (endoplasmic reticulum Ca²⁺ sensor) and Orai-1 (Ca²⁺ channel). Genetic depletion in hematopoietic cells of STIM1 or Orai-1 did also not reduce the INF/AAR ratio (167) hinting at alternative platelet activation pathways.

The final step of platelet activation is their aggregation and is mainly driven by α IIB β 3 activation. The inhibition of this integrin via Fab anti- α IIB β 3 administration did not alter the INF/AAR ratio when compared with control mice (167). In another study, α IIB β 3 inhibition by abciximab seems to reduce myocardial injury in isolated rat hearts through a reduction of platelet adhesion to the endothelium or leukocytes (179). This discrepancy can be probably explained by the different experimental models which can have a different impact on platelet activation. Platelet mechanisms in the MI model have been summarized in **Table 3A** and **Figure 2**.

In humans, several clinical trials tested platelet inhibitors in the context of MI. Currently, guidelines for the management of NSTEMI-ACS and STEMI patients recommend aspirin intake for long-term treatment for all patients without contraindications (148, 155, 156). Moreover, the dual antiplatelet treatment (DAPT) composed of aspirin plus a P2Y₁₂ inhibitor is recommended. Indeed, the CURE (Clopidogrel in Unstable Angina to Prevent Recurrent Events) trial was designed to assess the efficacy of the combination of aspirin and clopidogrel compared to aspirin alone (184). Patients with NSTEMI-ACS were enrolled within 24 h after symptom onset, and either treated with the combination treatment or aspirin alone. The DAPT significantly reduced the composite rate of death from cardiovascular causes, non-fatal MI and stroke. The rate of each component of this composite outcome also tended to be lower in the DAPT group. However, the risk of major bleeding is increased among patients treated with clopidogrel. TRITON-TIMI-38 (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel—Thrombolysis in Myocardial Infarction-38) is a phase III trial which enrolled patients with ACS (NSTEMI-ACS and STEMI) within 72 h after symptom onset (185). This trial was designed to compare the efficacy of prasugrel with clopidogrel, both associated with aspirin. The percentage of non-fatal MI (Clopidogrel: 9.5%; Prasugrel: 7.3%) and stent thrombosis (Clopidogrel: 2.4%; Prasugrel: 1.1%) was significantly reduced in patients treated with prasugrel, but it caused more life-threatening bleedings than clopidogrel treatment (from 0.9 to 1.4%). In conclusion, this trial showed that prasugrel is more effective at preventing ischemic events than clopidogrel in patients with NSTEMI-ACS

or STEMI. However, this beneficial effect is accompanied by an increased rate of major bleeding. The PLATO (Study of Platelet Inhibition and Patient Outcomes) clinical trial aimed to determine whether, combined to aspirin, ticagrelor is more efficient than clopidogrel in patients with ACS, enrolled within 24 h after symptom onset (186). Patients receiving ticagrelor had a significantly lower MI event rate (5.8%) compared to clopidogrel-treated patients (6.9%). The rate of death from any cause is also significantly lower for patients treated with ticagrelor (ticagrelor: 4.5%; clopidogrel: 5.9%). It is important to note that no difference in life-threatening bleeding was observed between the two treatment groups (ticagrelor: 5.8%; clopidogrel: 5.8%). This trial found that, in patients who have an NSTEMI-ACS or STEMI, treatment with ticagrelor compared to clopidogrel significantly reduced the rate of death from vascular causes, myocardial infarction, or stroke without an increase in the rate of overall major bleeding.

These results were later confirmed by the PEGASUS-TIMI-54 (Prevention of Cardiovascular Events in Patients With Prior Heart Attack Using Ticagrelor Compared to Placebo on a Background of Aspirin–Thrombolysis In Myocardial Infarction 54) trial which enrolled patient who had a MI 1 to 3 years earlier (187). Patients either received ticagrelor plus aspirin or aspirin alone. The ticagrelor treatment significantly reduced MI event rate (from 5.25 to 4.53%) (188). Moreover, this protective effect is consistent over time and this trial supports the use of prolonged therapy in patients who continue to tolerate this antiplatelet agent (189). Current guidelines recommend delivering DAPT to NSTEMI-ACS and STEMI patients, with aspirin plus ticagrelor or prasugrel (155). Clopidogrel can be administered to ACS patients who cannot receive the two previous antiplatelet agents.

Vorapaxar, a PAR₁ inhibitor, has been tested in clinical trials. The TRA 2P–TIMI 50 (Thrombin Receptor Antagonist in Secondary Prevention of Atherothrombotic Ischemic Events—Thrombolysis in Myocardial Infarction 50) clinical trial enrolled patients who had a history of atherosclerosis within the previous 2 weeks to 12 months (190). Patients were randomly assigned to either vorapaxar treatment or placebo. All concomitant medical therapy, including the use of other antiplatelet agents, was managed by the clinicians who were responsible for the care of the patients. Patients receiving vorapaxar had a reduced rate of MI event (from 6.1 to 5.2%) but presented an increase in major bleeding (from 11.1 to 15.8%). This trial assessed that inhibition of PAR-1 with vorapaxar reduced the risk of cardiovascular death or ischemic events in patients with stable atherosclerosis. However, it increased the risk of moderate or severe bleeding, including intracranial hemorrhage. The findings of TRA 2P–TIMI 50 clinical trial were confirmed by the TRACER (Thrombin Receptor Antagonist for Clinical Event Reduction in Acute Coronary Syndrome) trial. Investigators aimed to compare vorapaxar administration with placebo, in addition to standard therapy, in patients suffering from NSTEMI-ACS (191). The main result observed is a decreased rate of MI event for patients treated with vorapaxar. These data support the use of vorapaxar in MI secondary prevention since it provided net clinical benefit in patients at low risk for bleeding but high risk for ischemic events, as it especially prevented stent thrombosis after MI. However, the safety and efficacy of vorapaxar in STEMI patients have not been

TABLE 3 | (A) A comprehensive analysis of platelet mechanisms in myocardial infarction (MI) mouse model and **(B)** in MI patients (↓ decrease, ↑ increase, = no effect on MI or bleeding risk).

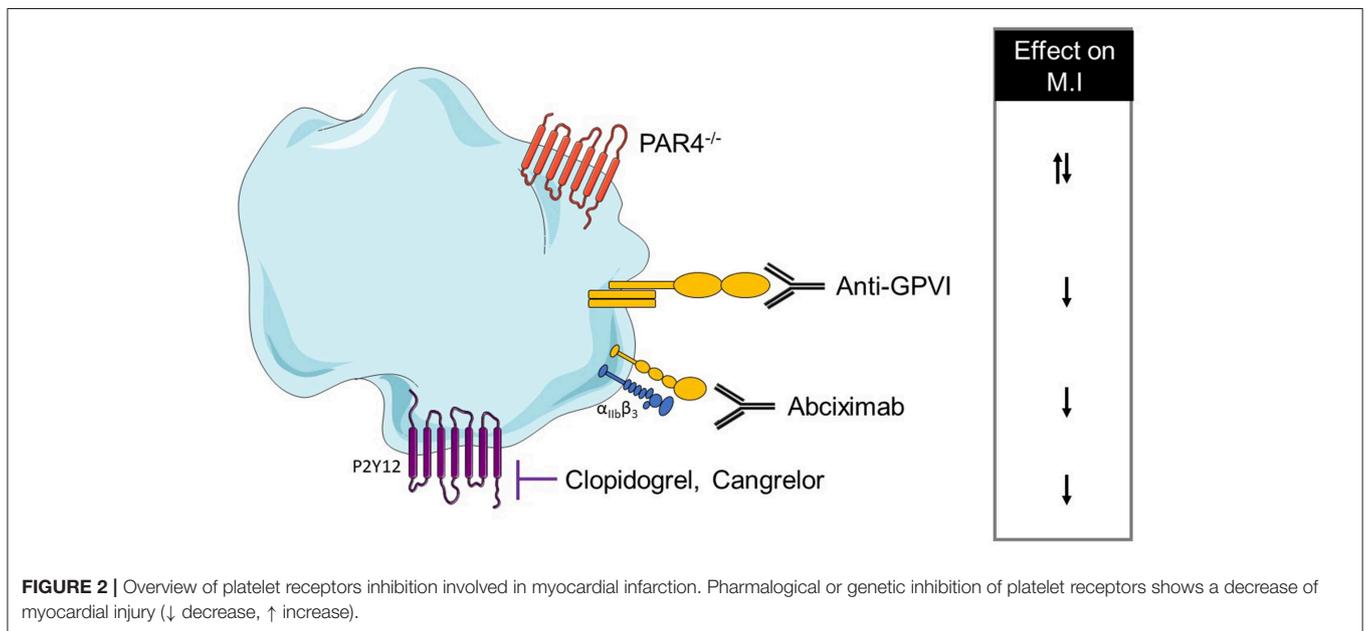
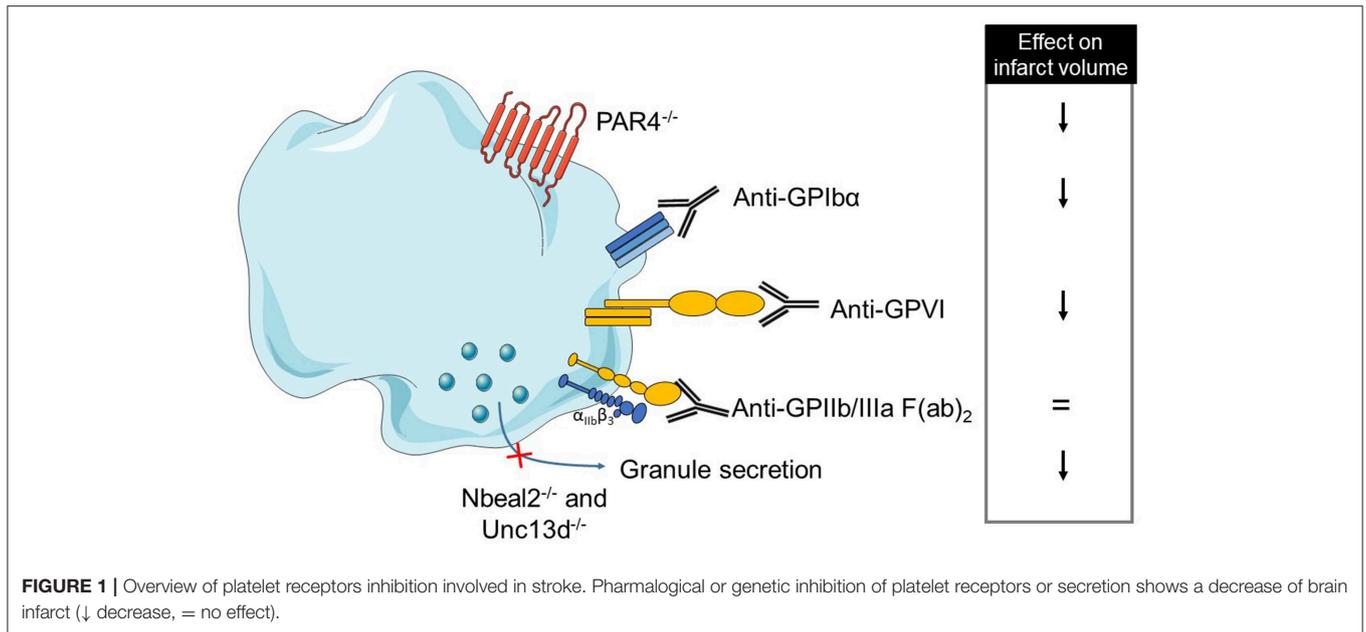
A				
Molecule targeted	Animal model	Effect on M.I.	References	
Platelets	Thrombocytopenia	↓	(166)	
GP1b α	Fab anti-GP1b α	=	(167)	
Pld1	Pld1 $^{-/-}$	=	(167)	
GPVI	Antibody anti-GPVI	↓	(167, 169)	
	Soluble GPVI-Fc	↓	(169)	
FcR γ	FcR $\gamma^{-/-}$	↓	(168)	
CLEC-2	Fab anti-CLEC-2	=	(167)	
PAR4	PAR4 $^{-/-}$	↑	(176)	
	PAR4 $^{-/-}$	↓	(177)	
	PAR4 antagonist	↓	(178)	
G α_{12}	G $\alpha_{12}^{-/-}$	↓	(124)	
α IIb β 3	Fab anti-GP1Ib/IIIa	=	(167)	
	Isolated hearts and perfusion of platelets from acute MI patients + Abciximab	↓	(179)	
δ granules	Nbeal2 $^{-/-}$	=	(167)	
Dense granules	Unc13d $^{-/-}$	=	(167)	
Stim1	Stim1 $^{-/-}$	=	(167)	
Orai1	Orai1 $^{-/-}$	=	(167)	
TXA $_2$	Aspirin	=	(165, 180)	
P2Y12	Clopidogrel	↓	(165, 166)	
	Isolated hearts and perfusion of platelets from acute MI patients + Cangrelor	↓	(179)	
B				
Molecule targeted	Clinical trial	Effect	Bleeding	References
P2Y12	CURE Clopidogrel + aspirin vs. aspirin alone	↓	↑	(184)
	TRITON-TIMI-38 Prasugrel vs. clopidogrel (+ aspirin)	↓ Prasugrel	↑ Prasugrel	(185)
	PLATO Ticagrelor vs. clopidogrel (+ aspirin)	↓ Ticagrelor	=	(186)
	PEGASUS-TIMI-54 Ticagrelor + Aspirin vs. Aspirin alone	↓	↑	(187–189)
PAR1	The TRA 2P-TIMI 50 Vorapaxar vs. placebo (+ concomitant medical therapy)	↓	↑	(190)
	TRACER Vorapaxar vs. placebo (+ standard therapy)	↓	↑	(191)

investigated yet. These clinical findings have been summarized in **Table 3B**.

PLATELETS PARTICIPATE TO THE DEVELOPMENT OF THE ABDOMINAL AORTIC ANEURYSM (AAA)

Abdominal aortic aneurysm (AAA) is a permanent and irreversible localized dilatation of the infrarenal segment of the

abdominal aorta caused by the degradation and remodeling of the layers of the vessel wall and a chronic wall inflammation. AAA can extend along the aorta (fusiform), or be localized (sacciform). Major AAA risk factors are age, atherosclerosis, hypertension, male gender, and smoking. In western countries, AAA incidence is ~0.4–0.67% annually and reaches 5–10% of men and 1% of women over 65 years old (192). This pathology is mainly asymptomatic and aneurysm rupture leads to death. Endovascular and open repair of AAA remains the only effective treatments. Nevertheless, many pharmacological therapies are



still under investigation like statins, angiotensin receptor blockers and anti-platelets drugs (193).

AAA is characterized by chronic inflammation with a large degradation of elastin and collagen fibers. It results in the proteolytic activity of matrix-degrading proteinases including matrix metalloproteinases (MMPs) leading to aorta dilatation. Reduced vascular wall thickness and the lack of tissue repair are associated with vascular smooth muscle cells apoptosis. The adventitia neovascularization induces inflammatory cells (lymphocytes, neutrophils and macrophages) infiltration in the aortic vessel wall, maintaining a continuous level of inflammation. This process contributes to the intra-luminal thrombus (ILT) formation (194), which involves platelets and

coagulation activation. Overall, the ILT thromboinflammatory status contributes to the outward remodeling and eventually to the disruption of wall integrity (195, 196).

The ILT is structured in multilayers. In AAA patients, ILT is often organized in three layers—luminal (in contact with the blood), medial and abluminal (in contact with the wall). Luminal ILT layer is biologically active and enriched in platelets, neutrophils, red blood cells and a dense fibrin network (197). The ILT has also been shown to contain weak pathogens like *Porphyromonas gingivalis* (Pg) which contribute to leukocyte recruitment (198). On the opposite side, the abluminal layer has a marked fibrinolytic activity and contains few cells (199, 200). ILT evolution can lead to vessel wall weakness due to the

high concentrations of reactive oxygen species (ROS), proteases and cytokines. Indeed, a study showed that ILT thickness is correlated with AAA diameter and MMP9 expression (201). The main specificity of the ILT in AAA is its non-healing property. The continuous release of neutrophil-derived proteases from these thrombi prevents vascular healing. Indeed, the re-endothelialization and adherence of mesenchymal stem cells are prevented by neutrophil proteases (202). This protease-rich thrombus is considered as the driving force in vessel wall rupture leading to death (203). However, ILT formation mechanisms in AAA are so far unknown.

Observational studies based on human tissue samples from AAA patients provide information at the late stage of the disease. In order to understand the mechanisms in the early steps, different animal models have been used, including mice and rats. The role of platelets and coagulation in ILT formation during AAA has been recently reviewed elsewhere (194). To study the role of platelets in AAA, two major models were used in mice and rats. The hypertension model, induced by angiotensin II in ApoE^{-/-} or Ldlr^{-/-} mice, reproduces important features of human AAA with inflammation, smooth muscle cells apoptosis and macrophage infiltration. However, aneurysms formed have a suprarenal location and abluminal thrombus formation occurs after an aortic dissection due to a false channel (204).

TABLE 4 | (A) A comprehensive analysis of platelet mechanisms in abdominal aortic aneurysm (AAA) animal models and **(B)** in AAA patients (↓ decrease, ↑ increase, = no effect on intraluminal thrombus ILT or aneurysm diameter).

A			
Molecule targeted	Animal model	Effect on ILT	References
αIIbβ3	Xenograft rats model + abciximab	↓	(197)
P2Y12	Xenograft model + AZD6140	↓	(209)
	Angiotensin II + Clopidogrel	↓	(210)
P2Y12 and TXA ₂	Angiotensin II + Clopidogrel and Aspirin	↓	(211)
B			
Molecule targeted	Clinical trial	Effect on aneurysm diameter	References
TXA ₂	Aspirin	↓ (between 40 and 49 mm)	(212)
P2Y12	Ticagrelor	=	(213)

The main model in rats consists of elastin degradation via elastase perfusion and presents the same characteristics as the first model. However, in this model, aneurysms have an infrarenal location and do not present a thrombus and hypertension (205). This model was also developed in mice (206). A recent study showed that administration of beta 3-aminopropionitrile fumarate salt (BAPN, inhibitor of lysyl oxidase) in the drinking water of elastase-treated mice resulted in ILT formation (207). Both of these models (elastase and angiotensin II) do not recapitulate all human characteristics but they contribute to better understand the disease.

A xenograft rat model which consists of grafting decellularized aorta of guinea pig into rat aorta has been shown to present an ILT (208). With this model, abciximab treatment (platelet aggregation inhibitor) reduces the aneurysmal diameter and ILT activities accompanied by fewer P-selectin expression and reduced vessel wall degradation. These results suggest that platelets are involved in the thrombus biological activity and aneurysm development (197). Similar results were observed in rats after 10 and 42 days of AZD6140 treatment, a P2Y12 receptor antagonist. A reduced ILT was observed as well as decreased MMP-9, MMP-2 expression, leukocyte infiltration, media and elastin preservation (209).

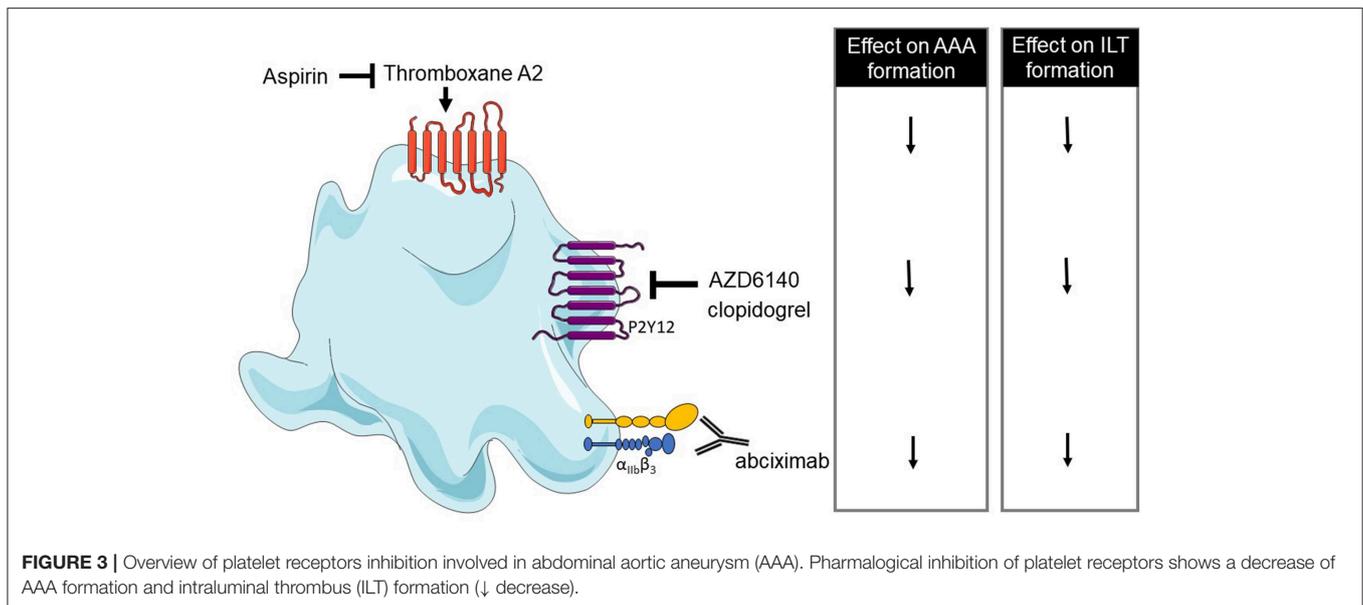


FIGURE 3 | Overview of platelet receptors inhibition involved in abdominal aortic aneurysm (AAA). Pharmacological inhibition of platelet receptors shows a decrease of AAA formation and intraluminal thrombus (ILT) formation (↓ decrease).

Other studies with angiotensin II mouse model have shown that clopidogrel treatment (inhibitor of P2Y₁₂), or aspirin (inhibitor of COX-2) reduces the macrophage infiltration, MMP2 and ROS production, suggesting that platelets play a role in vascular inflammation during AAA progression (210, 211). A reduction of thrombi, uPA, t-PA, and PF4 in the aorta was also observed in aspirin or clopidogrel-treated mice but these treatments have no effect on aorta diameter on established AngII-aneurysm model (211). However, clopidogrel administration in the early steps of AAA decreases the aorta diameter (210). The same treatments in patients emphasize that anti-platelet treatments can reduce AAA progression and rupture or dissection (211). Low-dose of aspirin can prevent the progression of AAA measuring from 40 to 49 mm and no decrease of AAA growth was observed in AAAs measuring <40 mm (212). The use of a P2Y₁₂ receptor inhibitor as ticagrelor treatment revealed a lack of difference in AAA size compared to placebo-treated subjects, suggesting that ticagrelor has no effect on the development of small AAAs. However, in this study, most of the patients did not present an ILT (213). These results have been summarized in **Table 4** and **Figure 3**.

Nowadays, there is no definite treatment to decelerate or stop AAA progression. Nevertheless, clinical and animal studies mentioned above provide additional information on the effect of platelets in AAA development, opening up treatment prospects that may be in the long term substitutes of heavy and invasive surgery.

CONCLUSIONS AND PERSPECTIVES

Over the past years, the field of platelets gained a lot of attention in their contribution to vascular diseases. Platelet biology is at the crossroads of several clinical specialties (cardiology, neurology,

pulmonology). The current use of antithrombotic drugs, aspirin, and P2Y₁₂ antagonists, is based on their inhibitory effect on platelet aggregation. While those drugs show a beneficial effect in CVD, they are still associated with some bleeding risks. Recent studies provided a new understanding of the role of platelets in vascular inflammation that extends beyond their role in aggregation. The development of future anti-platelet drugs will need to take into account the role of platelets in inflammation. In addition, as most of the thrombosis and inflammatory studies are conducted in healthy vessels, it is important to keep in mind that the identified mechanisms need to be validated in models relevant to CVD. Importantly, there is significant inter-individual variability of antiplatelet therapy responses among patients with CVD highlighting the need for tailored therapies to each individual. Central to this approach is the development of robust assays that can determine platelet reactivity in a patient-specific manner.

AUTHOR CONTRIBUTIONS

HL did the literature research and wrote the first draft of the review. KY wrote a part of the manuscript. RM designed the tables. YB contributed to the idea of the manuscript, wrote parts of the manuscript, and provided critical feedback. All the authors reviewed the manuscript and approved the submitted version.

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