

## Review Article

# Platelets Pleiotropic Roles in Ischemic Stroke

Anyanwu C, Hahn M, Nath M, Li J, Barone FC, Rosenbaum DM and Zhou J\*

Department of Neurology, SUNY Downstate Medical Center, USA

\*Corresponding author: Zhou J, Department of Neurology, SUNY Downstate Medical Center, 450 Clarkson Ave, Brooklyn, NY 11203, USA

Received: August 10, 2016; Accepted: September 30, 2016; Published: October 03, 2016

## Abstract

Stroke, one of the leading causes of morbidity in the world is strongly influenced by the activities of platelets and the bioactive materials they transport. The ability of platelets to actively participate in immunologic, hemostatic and regenerative processes makes them unique. Platelets can also exert a far-reaching influence when activated, by releasing microparticles containing lipids, receptors, proteins and genetic material into circulation. Apart from their well-established role in hemostasis, platelets have been identified as key players in inflammation, angiogenesis and CNS repair. This review summarizes the functions of platelets, their role in stroke and highlight areas in which platelet microRNA and microparticles can be used as biomarkers to predict cerebrovascular disease and stroke risks.

**Keywords:** Angiogenesis; Inflammation; Microparticles; miRNAs; Platelets; Repair; Stroke; Thrombogenesis

## Platelets Basic Features

Platelets are small, circulating, anucleate cell fragments of megakaryocytes that are well known for their roles in hemostasis and thrombogenesis. The platelet membrane consists of the typical bilayer of phospholipids, along with membrane glycoproteins that interact with various ligands. These ligands can either be soluble (thrombin, ADP, etc.) or fixed within the vessel wall or on other cells [1]. Found in the cytoplasm, are the alpha and dense granules. Alpha granules are more prevalent, making up over 90 percent of the total granules in platelets. Contained within alpha granules, are large peptides that contribute to hemostasis and thrombosis (such as vWF, fibrinogen, platelet factor 4, thromboglobulins) and coagulation factors V, XI and XIII. In addition, adhesion molecules (such as GPIIb/IIIa, GPVI and P-selectin) involved in platelet wall interactions and proteins involved in inflammation, wound-healing and growth are all found within  $\alpha$ -granules. The dense granules in comparison have a higher concentration of substances that contribute to local vasoconstriction (serotonin) and the recruitment of other platelets (ADP and calcium) [1].

## Platelets in Hemostasis

In the normal physiological milieu, the endothelial cell (EC) layer of the vascular wall prevents the activation of platelets by secreting inhibitory mediator such as nitric oxide and prostacyclin (PGI<sub>2</sub>). The EC layer also acts as a physical barrier between platelets and collagen in the extra cellular matrix [2]. Disruption of this layer during vessel injury exposes the underlining tissue matrix, resulting in a direct interaction between platelets and collagen. vWF mediates this interaction by acting as a bridge between the exposed collagen and platelets. vWF binds to the GPIIb receptor on the platelet membrane and tethers the platelet to the vessel wall [2,3]. Once platelets have been captured from circulation, they are then activated. This leads to increased intracellular calcium within the plasma membrane structural changes in the platelets, production of thromboxane and degranulation [4,5]. Several agonists such as ADP, serotonin

and thromboxane A<sub>2</sub> released by already activated platelets recruit more circulating platelets and grow the hemostatic plug. GPIIb/IIIa receptors on activated platelet plasma membrane are crucial in platelet-platelet interactions. These receptors are also located on  $\alpha$ -granules and they are functionally inert in resting platelets. In inside-out signaling, platelet activation by agonist, results in the activation of GPIIb/IIIa receptors. Subsequent binding of ligands to GPIIb/IIIa initiates a series of intracellular signaling (outside-in) that leads to degranulation, stable adhesion and clot retraction. In low shear conditions, fibrinogen acts as a link between the two GPIIb/IIIa receptors on the plasma membrane of adjacent platelets. Platelet aggregation under high shear is mediated by vWF binding to GPIIb/IIIa [1,6].

## Platelets in Thrombo-Embolic Ischemic Stroke

Atherosclerosis has a predilection for sites on the arterial tree with low oscillatory and endothelial shear stress. The disease mechanism in atherosclerosis involves several interwoven steps of lipoprotein retention, inflammatory cell recruitment, foam cell formation, apoptosis and necrosis, smooth muscle cell proliferation and matrix synthesis, calcification, angiogenesis, arterial remodeling, fibrous cap rupture, and thrombosis [7]. Vascular endothelial damage results in retention of lipoproteins such as LDL in the subendothelial space. Over time, these lipoproteins become oxidized and are phagocytized by macrophages via scavenger receptors [8]. As oxidized cholesterol accumulate within them, macrophages develop into foam cells, while a mesh extracellular matrix forms around the growing atherosclerotic plaque [9]. Erosion caused by blood flow can lead to plaque rupture, distal embolization and subsequent stroke [7,10,11]. Notably, platelets themselves can contribute to plaque rupture by recruiting neutrophils, which secrete proteolytic enzymes that can destabilize the plaque [12]. Additionally, platelets can contribute to ischemia by releasing vasoconstrictors such as serotonin and thromboxane A<sub>2</sub> [13]. Hence, platelets not only augment the growth of an atherosclerotic lesion, they also worsen the impact of atherothrombosis.

## Platelets and Common Risk Factors of Stroke

Platelets are loaded with a vast array of procoagulant factors and receptors that can consequently lead to thrombosis and stroke. Some of the major risk factors for stroke include hypertension, diabetes, heart disease, atherosclerosis, hyperlipidemia and smoking. When blood vessels are injured by these risk factors, the cholesterol-rich build-ups (plaques) develop. These plaques line the blood vessels. Ischemic stroke is a consequence of thrombosis, atherosclerotic plaque rupture or embolism. Both of thrombus and embolus formation result from platelet activation, aggregation and thrombin-mediated fibrin generation via the coagulation cascade [14]. In addition to sealing the vessel to prevent bleeding, the clot blocks downstream blood flow resulting in a non-hemorrhagic ischemic injury.

## Platelets in Inflammation during Stroke

Platelets play a very complicated role in inflammation during stroke. Recent *in vitro* studies indicate that platelets can adhere directly to the endothelium when either the EC and/or platelets are activated by agents such as thrombin (from circulating prothrombin), ADP (from platelets), the cytokines IL-1 $\beta$ , TNF- $\alpha$  or TGF $\beta$ 1 [15]. Platelet  $\alpha$ -granules mediate inflammatory responses by secreting a wide range of chemokines and expressing adhesion receptors that facilitates interactions between endothelial cells and leukocytes [2]. The platelet integrin P-selectin interacts with and recruits leukocytes by binding to its ligand P-selectin glycoprotein-1 (PSGL-1) found on the surface of leukocytes. *In vitro* studies have shown preferential recruitment of monocytes by EC adherent platelets [16]. The presence of P-selectin and platelet secreted chemokines such as CCL5 (RANTES) and CXCL4 (Platelet factor -4, PF-4) have been shown to cause the increased adherence of monocytes to ECs on the vessel walls. The adhered platelets also stimulate endothelial cells to produce CCL2 (MCP-1) that stabilizes monocytes captured by P-selectin [17]. The activation of integrins  $\alpha$ IIB $\beta$ 3 which participates in adhesion and coagulation has inflammatory functions [18]. Clinical studies indicate that platelet activation, cell-cell interaction and signaling are central in inflammatory states such as sepsis, acute kidney injury, dermal inflammation, vascular injury, myocardial infarction and stroke [19].

## Platelets in Angiogenesis during Stroke

Angiogenesis is the process of new blood vessel growth from pre-existing vessels. [20]. This process is regulated by a balance between pro-angiogenic and anti-angiogenic factors. Pro-angiogenic cytokines (angiogenic factors) include vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF), insulin-like growth factor, angiopoietin-1 and metalloproteinases [21]. For example, VEGF is the most important angiogenic factor. While it is also expressed by leukocytes, it has been clearly shown that platelets are the main contributors of VEGF in serum. VEGF is a biologically and therapeutically important growth factor because it promotes angiogenesis in response to hypoxia, a common underlying factor in a wide variety of both physiological and pathological settings [22]. On the other hand, PF-4, plasminogen activator inhibitor, angiostatin and thrombostatin are strong endogenous inhibitors of angiogenesis [21]. It has been noted that platelets stimulates the formation of blood vessels, in

*in vitro* in the rat aortic ring model via VEGF and bFGF. Blocking of platelet factor-4 also promotes angiogenesis. A study which looked into the mechanism of action, found that subcutaneous injection of platelets in matrigel to a mouse resulted in an intensive migration of fibroblasts into the matrigel as well as formation of blood capillaries *de novo*. This finding indicates that platelets affect different stages of the angiogenic response with a trend towards a pro-angiogenic effect despite the presence of angiogenesis inhibitors such as platelet factor 4. While a concomitant effect of bFGF and VEGF seems to be essential for the entire process of vessel formation, PDGF is effective only at the migration stage [23].

## Platelet Derived Microparticles

Microparticles (MPs) are small (0.1-1 micron) fragments of plasma membrane that are released from cells under duress via exocytosis [24]. The concentration of MPs in the blood is strongly correlated with the level of cellular stimulation, activation and apoptosis. MPs function as a transport and delivery systems for bioactive molecules that participate in hemostasis and thrombosis, inflammation, malignancy, infection transfer, angiogenesis and immunity [24,25,26]. Platelet derived microparticles (PMP) have been identified as the most abundant MPs in the blood [27]. They serve as vehicles for platelet peptides, receptors and microRNA that influence several vascular, hemostatic and regenerative processes. PMPs are formed following platelet activation under the influence of strong physiological platelet agonists such as thrombin and collagen [28], exposure to complement inflammatory proteins [29] or with induction of apoptosis [30]. Multiple studies have demonstrated that platelets miRNA and PMPs can promote neural stem cell survival and differentiation [31,32]. Therefore, it is not surprising that PMPs are significantly elevated in small and large vessel cerebrovascular accidents and/or multi-infarct dementias [33]. PMPs also play a pathophysiological role in the increased incidence of cerebrovascular events in patients with prosthetic heart valves [34]. Interestingly as related to inflammatory changes, the exposure of platelet microparticles to phospholipase A2 results in release of arachidonic acid, which is subsequently metabolized by platelets to thromboxane A2 [35]. This results in transactivation of platelets and endothelial cells and promotes monocyte-endothelial cell interactions. In the clinical setting, a drop in PMPs was observed following targeted stroke therapy in patients. Therefore, monitoring PMP levels may be useful in determining the therapeutic efficacy of various drugs used in ischemic stroke and TIA [33,36].

## microRNA in Platelets and Stroke

microRNAs (miRNAs) are short, non-coding RNAs that repress protein expression through a variety of mechanisms, including protein degradation, inhibition of elongation, premature termination and/or inhibition of protein synthesis or sequestration of mRNA transcripts [37]. Despite lacking a nucleus, platelets have a large store of miRNA [38]. When activated, platelets can release PMPs containing miRNA into the circulation. While the exact role of platelet miRNAs remains largely unknown, recent studies suggest a potential use of these genetic parcels as biomarkers of cardiovascular disease and stroke [39]. Some examples follow the expression of microRNA (miR)-126 which is found predominantly in ECs and endothelial progenitor cells (EPCs). miR-126 has been considered a master regulator of physiological

angiogenesis. In vessel injury and/or hypoxia, miR-126 up-regulation activates EPCs and ECs and contributes to vascular healing and formation of new vessels [40]. Recent data indicates that serum levels of another micro-RNA miR-145 is significantly up regulated in acute ischemic stroke this making it a suitable biomarker for the same [41]. In diabetes mellitus patients, low platelet and plasma miR-223 and miR-146a expression is a risk factor for ischemic stroke [42]. Finally, in experimental animal models on cerebral ischemia/reperfusion injury, the neuroprotective effect of miR-106b-5p antagomir (a chemically engineered oligonucleotide that prevents specific micro-RNA binding to the blocking site on an mRNA molecule) is associated with its inhibition of apoptosis and oxidative stress, suggesting a potential therapeutic target for ischemic stroke [43]. Hence, platelet derived and circulating miRNAs can be important in ischemic stroke regulation mechanisms and might be potential therapeutic targets in the future.

## The Role of Platelets in CNS Repair after Stroke

Platelets serve as recruiters of leukocytes into injured and inflamed tissue by assisting their rolling, arrest and transmigration. Despite the presence of the blood brain barrier (BBB), platelets carry out the function of leukocyte recruitment within the CNS while simultaneously interacting with cells in the neurovascular complex. These recruited macrophages with the help of the resident microglia, remove myelin debris (a potent inhibitor of oligodendrocyte precursor cells) via phagocytosis. The phagocytic cells also secrete growth and neurotrophic factors that stimulate oligodendrocyte progenitor cells. This creates conducive conditions for remyelination to occur [44].

Platelets also play a part in the regulation of CNS regenerative processes by interacting with CNS stem/progenitor cells. Adult neural stem cells (NSCs) are undifferentiated self-renewing multipotent cells that reside in the subgranular zone (SGZ) in the dentate gyrus of the hippocampus and the subventricular zone (SVZ) of the wall of the lateral ventricles [44]. Oligodendrocyte progenitor cells (OPCs) represent the major cellular source for mature remyelination oligodendrocytes and are widely spread throughout the CNS. Adult neural stem cells and oligodendrocyte progenitor cells contribute to brain cellular turnover, myelination and CNS repair. Brain derived neurotrophic factor (BDNF) is a member of the nerve growth factor family that is expressed in the central and peripheral nervous system. It plays an important role in CNS development, neurogenesis, and neuronal survival. It is synthesized by astrocytes and stored peripherally by platelets. Platelets carry greater than ninety percent of blood BDNF [45]. Importantly, activated platelets have been shown to accumulate around the vasculature of the stem cell niche in the SVZ of the lateral ventricles and stimulate the resident neural stem/progenitor cells resulting in their enhanced survival [44]. This increased survival effect appears to be due to the high levels of BDNF contained in released  $\alpha$ -Granules of activated platelets. One of the responses of reactive astrocytes to white matter damage secondary to prolonged cerebral hypoperfusion is the increased production of BDNF which activates repair processes and can accelerate endogenous oligodendrogenesis (e.g., increased mature myelinating oligodendrocytes from OPCs to reduce white matter damage [46]. For this reasons, platelets have an important role in modulating CNS repair in injury and stroke.

## Conclusion

The multifaceted features of platelets play a central role in both cerebrovascular disease pathogenesis and healing. As the platelet paradigm continues to expand, an increased understanding of the role platelets play in stroke can result in unique opportunities to develop effective drugs and new biomarkers for stroke.

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