Platelet Implication in Atherosclerosis Pathogenesis

Anastasia V. Poznyak [1*†], Varvara A. Orekhova [1], Vasily N. Sukhorukov [1], Alexandra A. Melnichenko [2†], Elizaveta M. Pleshko [2], Alexander N. Orekhov [1]

Abstract

Atherosclerosis is a prevalent cardiovascular disease that leads to serious complications like myocardial infarction and ischemic stroke. Platelets play a pivotal role in thrombosis and contribute to the development of atherosclerosis through interactions with the cellular environment. Consequently, platelets have emerged as a potential therapeutic target. This review explores the normal functioning of platelets and their involvement in atherosclerosis progression, highlighting their participation in inflammatory responses within the arterial wall. Platelets activate and release mediators that promote vascular inflammation and endothelial dysfunction, key features of atherosclerotic plaque formation. They also interact with circulating immune cells, exacerbating the inflammatory milieu and fostering disease progression. Targeting platelets presents a promising approach for therapeutic interventions in atherosclerosis. Antiplatelet agents aim to impede platelet activation and aggregation, reducing thrombosis risk. Novel strategies that target platelet interactions with inflammatory cells and modulate platelet-derived inflammatory mediators are also being investigated. Further research is needed to fully exploit the potential of platelet-targeted therapy. Understanding the precise role of platelets at different stages of atherosclerosis and their interactions with immune cells and the inflammatory milieu will enhance our understanding of disease pathogenesis and guide the development of more effective therapeutic approaches. In conclusion, platelets significantly influence atherosclerosis by contributing to thrombus formation and promoting inflammatory processes. Recognizing platelets as a therapeutic target opens up new possibilities for mitigating the consequences of atherosclerosis and improving patient outcomes.

Keywords: Platelet; Atherosclerosis; Foam cells; Inflammation; Thrombosis.

Significance | Platelets reduce cardiovascular risks, are important in atherosclerosis, and may be a therapeutic target.

*Correspondence: Anastasia V. Poznyak and Alexander N. Orekhov
Institute for Atherosclerosis Research,
Osennyaya 4-1-207, 121609 Moscow, Russia
E-mail: tehhy_85@mail.ru (A.V.P.);
alexandernikolaevichorekhov@gmail.com
(A.N.O.)

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1. Introduction

Platelets, which are enucleated cells derived from megakaryocyte fragmentation in the bone marrow, have long been acknowledged for their pivotal involvement in primary hemostasis and the healing process of vascular damage. However, recent research has unveiled their participation in various pathological conditions such as cardiovascular disease (CVD), immunological and oncological processes, and other diseases.

Under physiological circumstances, circulating platelets assume a quiescent discoid shape in close proximity to the apical surface of endothelial cells, thanks to the anti-adhesive properties of healthy endothelial cells. This delicate equilibrium is maintained through the actions of platelet inhibitors, fibrinolysis activators, and coagulation inhibitors secreted by the endothelial cells. Nevertheless, when the endothelium suffers damage, platelets undergo activation and adhere to the exposed subendothelial tissue, resulting in their morphological transformation into an

Author Affiliation:
[1] Institute for Atherosclerosis Research, Osennyaya 4-1-207, 121609 Moscow, Russia;
E-mail: tehhy_85@mail.ru; varvaraao@gmail.com; vnsukhorukov@gmail.com, alexandernikolaevichorekhov@gmail.com
[2] Laboratory of Angiopathology, Institute of General Pathology and Pathophysiology, 8 Baltiiskaya Street, Moscow 125315, Russia;
E-mail: sasha.melnichenko@gmail.com; lizapleshko@yandex.ru
†These authors contributed equally.

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irregular shape accompanied by pseudopod formation. This activation process involves the secretion of various bioactive molecules stored in platelet granules, subsequently triggering additional platelet recruitment and activation.

Platelet granules contain an assortment of molecules, including adenosine diphosphate (ADP), adenosine triphosphate (ATP), serotonin, histamine, epinephrine, calcium, fibrinogen, fibronectin, and growth factors, among others. These molecules play crucial roles in platelet aggregation, activation of coagulation factors, and clot retraction, culminating in the formation of a stable platelet plug and tissue healing.

While platelets have conventionally been associated with hemostasis, emerging evidence suggests their implication in atherogenesis and inflammatory processes. Even in the absence of endothelial denudation, platelets have the ability to adhere to the endothelium, contributing to the development and progression of atherosclerotic lesions. They engage in interactions with immune cells, release microparticles and granule proteins, and interact with modified low-density lipoprotein in the atherosclerotic environment.

Comprehending the inflammatory predisposition of platelets is vital for unraveling their role in atherosclerosis. Although platelets remain largely quiescent under physiological conditions, their activation status becomes heightened in patients with advanced cardiovascular disease. It remains unclear whether platelets contribute to lipid retention in the arterial wall, leading to atherosclerotic plaque formation, induce asymptomatic thrombus formation and secrete inflammatory mediators, or if their inflammatory functions arise from chronic interactions with leukocytes and endothelium.

This comprehensive review aims to explore the intricate relationship between platelets and atherosclerosis, with particular emphasis on platelet-mediated inflammation. It will delve into the mechanisms underlying platelet activation, adhesion, and aggregation during thrombus formation, as well as the subsequent interplay between platelets, leukocytes, and endothelial cells. Furthermore, it will investigate the potential of anti-inflammatory treatments targeting platelets for the prevention and management of atherosclerosis.

By shedding light on the multifaceted roles of platelets in atherosclerosis and inflammation, this review aspires to provide valuable insights for the development of future therapeutic approaches that selectively target platelet-mediated inflammation while preserving their indispensable hemostatic functions.

Normal functioning of platelets

Platelets are enucleated cells that are both structurally and metabolically complex and formed in the bone marrow resulting the fragmentation of megakaryocytes. The roles of platelets in cardiovascular disease (CVD), immunological and oncological processes and other diseases has newly appeared in addition to already defined role of platelets in primary hemostasis, the important part of vascular damage healing process (Huilcaman et al., 2022).

Prevention of blood loss from injured vessels is carried out by components of the coagulation system along with platelets. Partly due to the anti-adhesive characteristics of quiescent endothelial cells circulating platelets near the apical surface of endothelial cells stay in quiescent discoid state as well, not forming stable adhesion contacts, this balance remains while the endothelium is healthy and not impaired and the blood flow is laminar (de Boer et al., 2006). Numerous factors are required for endothelial cells to have these antiadhesive characteristics, among them are synthesis and secretion of platelet inhibitors, fibrinolysis activators, coagulation inhibitors, as well as the proximity of neutral phospholipids and negatively charged heparin-like glycosaminoglycans. When the vessel is damaged, platelets start their activation and adhesion to the endothelium resulting their exposure to exposed to collagen, von Willebrand factor (VWF), laminin, thrombospondin, and other highly thrombogenic molecules present in the subendothelial tissue (Yau et al., 2015). At the subcellular level an increased concentration of intracellular calcium leads to platelet activation. The platelet surface becomes irregular because of the formation of pseudopods, which is the result of the centralization of platelet granules and reorganization of the actin cytoskeleton (Shin et al., 2017). After the content of platelet granules is released, the adhesion process starts, platelets release the stored bioactive molecules that can induce the activation and recruitment of other cells with a mechanism of highly regulated exocytosis. There are two types of platelet granules: α granules that consist of fibrinogen, fibronectin, P-selectin, factor V, factor VIII, platelet-derived growth factor (PDGF), platelet factor 4 (PF4 or CXCL4) and tumor growth factor-α (TNF-α), and δ granules consisting of adenosine diphosphate (ADP), adenosine triphosphate (ATP), serotonin, histamine, epinephrine and calcium. A surface for the combination of different coagulation factors is provided by calcium and phospholipids released from the granules (Golebiewska and Poole, 2015; Di Minno et al., 2020). The other molecules trigger a secondary wave of platelets activation, thromboxane A2 (TxA2) is thereafter synthesized by the activated platelets, then promotes platelet aggregation, increases the activation signals, and establishes positive feedback. To close the injury temporarily at initial platelet plug is formed as a result of the platelet aggregation that is stimulated by the TxA2 and ADP coactions, while the latter also promotes in the platelets a conformational change in integrin αIIbβ3 (or glycoprotein IIb/IIIa) through "inside-out" signaling, leading to altering the integrin state to high-affinity or open from low-affinity or closed one (Rucker and Dhamoon, 2022; Sangkuhl et al., 2011). This

allows the binding sites to be exposed by the integrin αIIbβ3 to integrin ligands like fibrinogen and VWF and induce platelet activation and adhesion. Regulation of the cytoskeleton reassembly is provided by the "outside-in" signaling that follows these changes and enables to form a stable aggregate of platelets and clot retraction. Platelets can take part not only in hemostasis, but they are also especially relevant in tissue regeneration in the first stages of the repair (Durrant et al., 2017).

Platelets in atherogenesis

There is much evidence proving that platelets are involved in early stages of atherogenesis as well as in atherothrombotic complications, being able to attach to the endothelium even without endothelial denudation under various pathological conditions. The atherosclerotic lesions develop and progress under the influence of platelets releasing microparticles and granule proteins, cross talking with endothelial and immune cells, and interacting with multiple modified low-density lipoprotein (Wu et al., 2017; Myasoedova et al., 2018). In Figure 1, we depicted the simple scheme of the main mechanisms of atherogenesis, that are implemented via platelets.

From thrombus to inflammation

It is important to understand platelets elementary biology so as to acknowledge their inflammatory propensity. Platelets are circulating at rest under physiological conditions, and quiescent platelets do not form PLAs and do not express CD40L or P-selectin (Cognasse et al., 2022). Though platelet activation status gets higher when a patient is suffering from advanced cardiovascular disease. How enhanced platelet activation affect initial lipid retention in the arterial wall progressing to clinical manifestations of atherosclerotic plaque formation is yet to be discovered. It is not understood yet if platelets over time induce numerous asymptomatic thrombi, secrete the inflammatory mediators therein and contribute to the leukocyte recruitment, or whether the inflammatory functions of the platelet are exerted due to chronical interaction with leukocytes and endothelium (Linton et al., 2019; Poggio et al., 2014).

There are four stages in the mechanism of thrombus formation: tethering of platelets, activation and strong adhesion, platelet recruitment and aggregation, and the last stage – thrombus stabilization. A component of the GPIb-V-IX complex glycoprotein (GP) Ibα is expressed constitutively on platelets, mediates platelet tethering and binds to collagen bound von Willebrand factor (VWF), triggering platelet adhesion (Rivera et al., 2009; Perrot et al., 2020). GPVI is the main agonist for initial activation of platelets and granule release, GPVI binds to collagen causing platelet activation. All major steps of thrombus formation – platelet tethering, firm adhesion, and aggregation – include GPVI-collagen interactions, as demonstrated Massberg et al (Massberg et al., 2004). At the location of vascular damage thrombin generates quickly and causes integrin activation, change of the shape and granule secretion, being the most potent activator of platelets. After the platelets are firmly attached, the content of their granules, such as adenosine diphosphate (ADP) and thromboxane A2 (TXA2), is released and spread, stimulating activation, which leads to the integrin αIIbβ3 conformational change and an increase of its affinity for fibronectin, fibrinogen and VWF (Becker et al., 2018). The central molecule for aggregation and stabilization of platelets is αIIbβ3 in active form. CD40 ligand (CD40L) and growth-arrest-specific gene 6 (GAS6) are present in the platelet-platelet synapse and take part in the process of platelet activation, enhancing stabilization. As it was showed lately by Ho-Tin-Noe et al. in a spontaneous tumor hemorrhage murine model, tumor necrosis factor (TNF)-α may induce leukocyte recruitment, that is involved in bleeding during thrombocytopenia (Huang et al., 2019; Kojok et al., 2018). In addition, neutrophils depletion leads to TNF-α being unable to provoke bleeding in thrombocytopenic mice, thereby, inflammation leads to increased crosstalk between leukocytes, platelets and endothelial cells by changing the settings of the anticoagulants and procoagulants significantly. The atherosclerosis formation is considered to be a disease mostly driven by leukocytes, thus there is a need for investment in studying of the platelet's effects in atherosclerosis development and the leukocyte role in formation of thrombus, also a process driven by platelets (Ghasemzadeh et al., 2022; Sainger et al., 2013; Kapoor et al., 2018).

Inflammation

New information on the potential of anti-inflammatory treatments for cardiovascular prevention was discovered by the CANTOS in connection with the atherosclerosis inflammatory genesis, Ridker et al. (Ridker, 2019) demonstrated a treatment with a monoclonal antibody targeting IL1β, named Canakinumab, of 10,061 patients with a history of myocardial infarction, which led to significant decrease of cardiovascular events. In a mouse model of atherosclerosis, the IL-1β atherogenic effect was already showed, and antibody administration was linked with a reduction of inflammatory biomarkers IL-6 and C-reactive protein (CRP), though another trial (CIRT) did not demonstrate the same effect by using methotrexate at low dosage (Ridker et al., 2019). Low-dosage methotrexate treatment did not lead to a comparable impact on IL-6 and CRP, so the anti-inflammatory therapy choice may not have been appropriate for the task at hand. The decrease in cardiovascular diseases in patients treated with colchicine in low doses for secondary prevention (COLCOT) could be confirmed by a trial published lately, though this treatment was associated with more infections because of the colchicine and Canakinumab immunosuppressive effects (Bouabdallaoui et al., 2020).

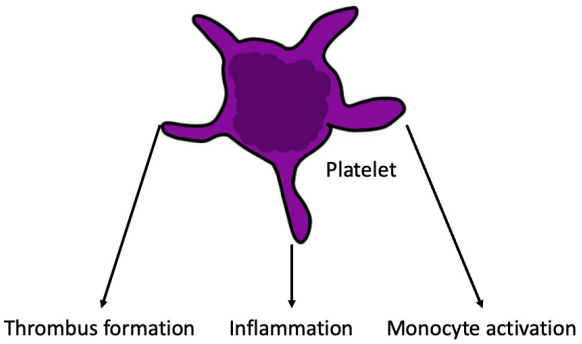

Figure 1. Main mechanisms, through which platelets are involved in atherogenesis.

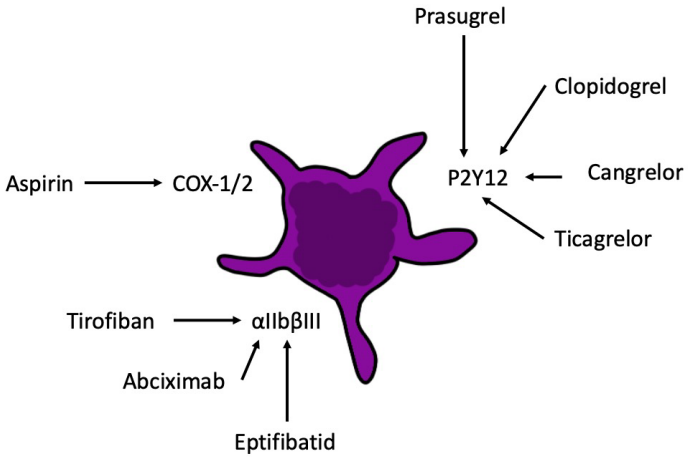

Figure 2. Main antiplatelet drugs and their targets.

Platelets could be another way of inflammation inhibition in the context of atherosclerosis. In clinical settings and animal studies multiple reviews have concentrated on the marked anti-inflammatory effects that could be caused by antiplatelet therapy. Furthermore, a number of scientists state that platelets should be referred to as immune cells because of their inflammatory functions (Müller et al., 2015). Aspirin and Clopidogrel in animal models displayed inhibitory effects on initiation and progression of lesions along with decreased vascular inflammation, as evidenced by decreased NFκB activity, noticeably reduced serum levels of inflammatory markers and plaque phenotype become more stable. In patients with coronary artery disease (CAD) aspirin lowered proinflammatory markers levels and resulted in positive effect on endothelial dysfunction (Migdalski and Jawien, 2021). P-selectin surface expression and integrin activation are linked with platelet activation by Clopidogrel, thereafter, in patients with CVD or diabetes Clopidogrel treatment demonstrated significant reductions in monocyte integrin expression and platelet P-selectin, formation of platelet-leukocyte aggregate and inflammatory markers serum levels (Storey et al., 2002; Pluta et al., 2022). So, in patients with diabetes withdrawal of Clopidogrel led to a considerable increase in thrombotic and proinflammatory properties. Moreover, extracellular vesicles release from platelets involved in thrombotic and inflammatory processes are inhibited by Ticagrelor and Clopidogrel (Angiolillo et al., 2006).

Generally, these data show anti-inflammatory characteristics of anti-platelet medicines, which mean there might be a role for targeting platelets in initial atherosclerosis prevention. Nevertheless, increased risk of bleeding caused by available anti-platelet drugs counteracts those anti-inflammatory properties. Further we would like to give some mechanistic explanations for that by concentrating on platelet-mediated inflammationю This study will tell how anti-platelet drugs might target platelet-mediated inflammation not impairing the platelets hemostatic functions (Warner et al., 2011).

Platelet-endothelium interactions

Unimpaired endothelial cells in physiological conditions demonstrate an antithrombotic phenotype and by surface expression of ectoADPase (CD39) and secretion of NO and prostacyclin (PGI2) suppress platelet activation. When endothelium is activated and inflamed, those control mechanisms are lost, and that defines what is called an endothelial dysfunction, a significant antecedent condition of atherosclerotic lesions (Hamilos et al., 2018; Branchetti et al., 2014). Platelets demonstrated noticeably increased adhesion to the atherosclerotic endothelium of carotid arteries in a mouse model of atherosclerosis, the process that mediates platelet adhesion to the endothelium includes two stages characterized by specific receptors. The primary contact is reversible, it is described as tethering or rolling on the endothelial surface. Platelet and endothelial selectins as well as their counterreceptors GPIb-IX-V and P-selectin glycoprotein ligand-1 (PSGL-1) mediate this contact (Prabhu et al., 2011). In mice with ApoE deficiency blocking mAb to GPIb inhibited the firm and transient adhesion to the vascular surface, although the deficiency was not related to desreaced formation of atherosclerotic lesions. After initial rolling of platelets on endothelial surfaces, endothelial β3-integrins and platelets mediate firm adhesion. Binding of platelet to endothelial cells was lowered following antibody blockade of endothelial receptors β3-integrin and ICAM-1 or αIIbβIII, αIIbβIII-binding proteins (fibronectin, fibrinogen and vWF) (Johnson, 2020; Di Minno et al., 2017). Moreover, firm adhesion in a mouse model of atherosclerosis was fully inhibited after loss of αIIbβIII, although patients with Glanzmann thromboasthenia with αIIbβIII or αvβIII deficiency demonstrated impaired hemostasis and no protection from atherosclerosis. Thus, firm adhesion is, inter alia, provided by platelet GPIb and αIIbβIII along with endothelial αvβIII and ICAM-1 interacting through a bridging mechanism that includes fibronectin, fibrinogen and vWF (Li et al., 2021). Platelet adhesion leads to platelet activation and formation of the basis for crosstalk of platelet and endothelium, which is mediated by ligand-receptor interaction, secreted factors and platelet-derived microparticles. Platelets, being anuclear, contain a set of 3000–6000 megakaryocyte-derived (pre-)mRNAs and can produce numerous proteins such as tissue factor, IL-1β etc. Soluble mediators (thrombin, collagen, ADP) and β3-integrins are modulating through outside-in signaling protein synthesis and platelet resident pre-mRNAs splicing (Saboor et al., 2013). It looks like the platelet transcriptome is associated with inflammation and atherothrombotic complications because expression of Toll-like receptor 2 mRNA showed higher level in patients suffering from coronary atherosclerosis than it did in patients without coronary syndrome. In addition, platelet expression of multiple inflammatory mRNAs is linked to levels of inflammatory biomarkers IL-6 and CRP, thus, platelet gene expression differential modulation might be an upcoming goal for forthcoming therapeutic approaches (Heger et al., 2019). Along with mRNAs, platelets represent the most important source of circulating microRNAs (miRNAs), which are noncoding, short RNA-molecules, regulating posttranscriptional gene expression and taking part in different atherosclerotic processes. It has been indicated that microRNAs contribute to metabolic and vascular disease. Moreover, as it has recently been revealed, the components required to assemble a functional inflammasome are present in platelets, where even IL1β pre-mRNA can be processed. Endothelial activation is stimulated by platelet-derived IL-1β,

microvesicle-associated or soluble, which causes upregulation of adhesion molecules (αvβIII-integrins, VCAM-1, ICAM-1) (Leng et al., 2022). Thus, endothelial cells become more adhesive for cells of other types and enhanced expression of proinflammatory chemokines/cytokines (IL-8, MCP-1, IL-6) by endothelial cells promotes. For instance, in the serum of patients with Systemic Lupus Erythematosus (SLE) there was shown IL1β-mediated gene expression of ICAM-1 and IL-8 in endothelial cells (Atehortúa et al., 2017). And naturally SLE is linked with a higher risk of premature cardiovascular events. In addition, MCP-1 promotes lesion formation being the main mediator of monocyte recruitment to atherosclerotic lesions. Platelets besides T-cells also express CD40L when activated (Mak and Kow, 2014).

In addition to CD40, various receptors for CD40L, such as and α5β1, αIIbβIII, and αMβ2 (Mac-1), have been identified. These receptors take part in different processes like hemostasis, thrombosis, leukocyte adhesion, atherogenesis and immunological functions. After platelet activation, CD40L moves to the cell surface and stimulates surface expression of adhesion molecules (VCAM-1, ICAM-1, E-Selectin) and the secretion of endothelial chemokines (MCP-1, IL-8). Hereby, platelet CD40L mediates leukocyte adhesion through platelet-endothelial and platelet-leukocyte interactions and thus contributes to the formation of atherosclerotic lesions in mice with hyperlipidemia (Hasan et al., 2022; Zanobini et al., 2018; Michel et al., 2017).

In various mouse models, failure of CD40L signaling of atherosclerosis indicates an inhibitory action on both atherogenesis and atherosclerotic plaques stabilization. Though, a clinical trial of a humanized anti-CD40L antibody using in patients with proliferative lupus nephritis was finished early due to thromboembolic complications. CD40L taking part in various physiological processes, such as immunological functions or hemostasis, demands specific inhibition of dysregulated and pathological reactions. Thus, different CD40L receptors can provide differential targeting for certain functions mediated by CD40L (Michel et al., 2017; Daub et al., 2020).

Lately in comparison with CD40L there has been also shown similar effects on platelet-leukocyte-interactions, endothelial activation and adhesion, atherogenesis and leukocyte recruitment for platelet CD40. In view of the broad expression of CD40/CD40L these data point to reciprocal CD40L-CD40 interactions among leukocytes, endothelial cells and platelets (Ramirez et al., 2010).

Vice versa, platelet functions and reactivity are modulated by endothelial cells, which upon activation Fractalkine (CX3CL1) is expressed on, binding to platelet CX3CR1 and triggering P-selectin-dependent platelet-leukocyte-interactions and platelet degranulation. There has been observed a 40% decrease in leukocyte adhesion in vitro as a result of disruption of the CX3CL1-CX3CR1 axis (Schulz et al., 2007).

Platelets attach to the endothelium together in a sequential process, which results in endothelial activation as well as an increased adhesiveness and secretion of cytokines, consequently, disrupted platelet-endothelial interactions can contribute to the inhibition of atherogenesis at initial stages (Bykov et al., 2022).

Platelet-leukocyte interactions

Platelets cooperate with immune cells and stimulate recruitment of leukocytes to inflamed endothelial sites. That is the main step in atherogenesis subsequent to the cytokine secretion or straight cell-to-cell interactions. There been suggested two different direct leukocyte recruitment mechanisms. The platelets either interact with leukocytes and form platelet-leukocyte-aggregates (PLA) before making contact with endothelium, or they attach to activated endothelium and bind leukocytes (Wang and Tang, 2020). As in vitro studies have indicated, the formation of PMA leads to an increase of monocyte adhesion and cluster formation on activated endothelial cells, and moreover, PLAs potentially are a therapeutic target being a sensitive marker for activation of platelets and myocardial infarction after plaque rupture (Dann et al., 2018). During platelet-endothelial crosstalk, platelets and leukocytes directly interact being mediated by a complex process, which involves the primary binding of platelet P-selectin to the leukocyte PSGL-1, causing integrin activation and cytoskeletal rearrangement. The platelet GPVI and extracellular matrix metalloproteinase inducer (EMMPRIN) interaction as well as platelets GPIbα, ICAM-1/2, JAM-3 and αIIbβIII with a fibrinogen bridge and leukocyte LFA- and 1 Mac-1 mediate the firm adhesion (Dole et al., 2007; Sainger et al., 2012).

Antibodies blocking PSGL-1/P-selectin have shown a strong inhibitory effect on monocyte adhesion to endothelium and platelet-monocyte interaction in vitro and thus appear to be a appropriate mechanism for next therapeutic approaches to prevent atherosclerosis, moreover, deficiency of P-selectin in platelets caused a decrease of platelet adhesion and atherosclerosis in mice with hyperlipidemia (Kappelmayer and Nagy, 2017). A monoclonal antibody against P-selectin Inclacumab reached quick inhibition of formation of PLA in healthy subjects and showed cardioprotective activity in NSTEMI patients going through percutaneous coronary intervention. Treatment was not supposed to have a higher incidence of adverse events such as bleeding or infections, hereby, current data support the potential therapeutic advantage of specific P-selectin inhibition. In addition, platelet P-selectin interact with sulfatides expressed on the surface of platelets as a counter-receptor, which causes an increase in their aggregation and stability of aggregates, formation of PLA after activation of αIIbβIII and increased expression of P-selectin, a positive feedback loop (Stähli et al., 2016; Parolari et al., 2016).

These findings may contribute to the prolonged bleeding time reported in P-selectin-deficient mice and link P-selectin to platelet hemostatic functions. Differential inhibition of PSGL-1- and sulfatide-mediated functions of P-selectin by peptide antagonists has been demonstrated recently by Korporaal et al. There is a promising approach for next therapeutic interventions in the specific distinction between hemostasis and the proatherogenic functions of P-selectin presents (Korporaal et al., 2019).

Monocytes are activated by stimulated platelets, which also promote a proinflammatory phenotype in macrophages derived from monocytes. That is linked with secretion of pro-inflammatory cytokines (IL-1, TNFα, MCP-1, IL-8) after NFκB. activation and increased adhesion to endothelial cells. Furthermore, there has been demonstrated TREM-1-dependent leukocytes activation, and a ligand for trigger receptor expressed on myeloid cells 1 (TREM-1) is expressed by human platelets (Fu et al., 2021). TREM-1, while not being particularly involved in the process of adhesion, supports atherogenesis by triggering and enhancing inflammatory reactions, the activation leading to an increase of foam cells production via CD36 activation and secretion of proinflammatory cytokines and MMPs. Pharmacological inhibition or deficiency leads to a noticeable decrease of atherosclerosis and thus provides a potent therapeutic target. In addition, EMMPRIN induces NFκB-dependent secretion of proinflammatory cytokines and may link monocyte activation and platelet adhesion. There is data showing that antibodies against P-selectin or PSGL-1 might in part inhibit a proinflammatory monocyte phenotype induction, which indicates that both secretion of soluble cytokines and direct intercellular interaction have influence on macrophage activation (Farahi et al., 2021).

Leukocyte recruitment is also mediated by platelets using a particular mechanism: the secretion of proteins that were stored in α-granules and then released after activation, i.e., CXCL4 (PF4), RANTES (CCL5) and IL-8 (CXCL8). There is no need in direct physical contact for the two cells in case of cytokine-driven activation of monocytes via platelets. Secretion and modulation of platelet activation can be a potential target for preventing atherosclerosis as well as regulating other platelet functions such as angiogenesis, as numerous studies suggest differential packaging and release of proteins stored in α-granules, i.e., VEGF, CXCL4, CXCL12 or endostatin, with platelet being activated by different agonists (Rossaint, et al., 2018).

The most abundant platelet chemokine CXCL4 demonstrates immunomodulatory and antiangiogenic functions while interacting with growth factors and proteoglycans or binding directly to CXCR3B and also induces atherogenesis beyond CXCL4-oxLDL interactions by promoting polarization of macrophages towards a pro-atherogenic phenotype called "M4", which were identified in human atherosclerotic plaques. Hereby, in two mouse models of atherosclerosis the deficiency of CXCL4 decreased formation of lesions and showed a potential prevention mechanism (Gleissner, 2012).

Firm adhesion and transmigration of monocytes across inflamed endothelium after immobilization on monocytic and endothelial surfaces if triggered by platelet-derived CCL5, which has also been found on the carotid arteries luminal surfaces in mice with ApoE-deficiency and atherosclerotic lesions after an activated platelets injection or wire-induced injury. In addition, there was recorded a 50% reduction in plaque formation in mice with hypercholesterolemia as a result of CCR1/5 CCL5 inhibition, which highlights the chemokine's proatherogenic activity (Evans et al., 2022). CXCL4 forms heterodimers with CCL5 and increases CCL5-mediated monocyte arrest on endothelial cells in addition to polarization of monocytes. Monocyte arrest on endothelial cells and atherosclerosis were significantly reduced in vitro and in vivo as a result of the specific inhibition of CXCL4-CCL5-interaction by a peptide inhibitor (MKEY/CKEY) in ApoE-deficient mice, which did not cause an immune functions impairment as opposed to the CCR1/5 chemokine receptors blockade. In addition, in mouse models of myocardial infarction and stroke MKEY decreased cardio- and neuroinflammation, improved the functional result and was also linked to a reduces size of infarct. According to these results, the inhibition of CXCL4-CCL5 interaction could be a potent mechanism for vascular inflammation primary prevention and targeted therapy (Dickhout et al., 2021).

Moreover, macrophage migration inhibitory factor (MIF) and chemokines CXCL12 (SDF-1) may also be promising targets for prevention, according to growing new evidence. Platelet-derived CXCL12 induces atherogenic conditions of insulin resistance and dyslipidemia, neointimal hyperplasia, vascular inflammation and angiogenesis and thus displays proatherogenic characteristics (Gried et al., 2010). Several monocytic functions, such as migration, survival, monocyte polarization and adhesion to activated platelets, are regulated by CXCL12, which is interacting with monocytes through CXCR4/7 and hereby may take part in monocyte recruitment. In addition, CXCL12 stimulates polarization of monocytes into predominantly CD163+ M2-like macrophages and induces formation of foam cells. Inhibition of CXCL12-signaling, considering its proatherogenic effect, can be a potential platelet-directed target for the atherosclerosis prevention (Chatterjee et al., 2015). Interference with homodimerization would modulate in vivo the binding of receptors of CXCL12 and thus might be an interesting approach. Additionally, formation of neointima in mice with atherosclerosis after carotid injury was repressed by CXCR4-inhibition, i.e., CXCL12-receptor blockade (Murad et al., 2021).

MIF is a platelet-derived chemokine that has a delayed secretion kinetics in comparison with CXCL12. MIF takes part in adhesion of monocytes to endothelial cells through CXCR4/2. In a murine model of atherosclerosis antibody blockade of MIF or MIF-deficiency induced high plaque stability while in vitro reducing monocyte adhesion to endothelial cells and strongly inhibiting monocyte chemotaxis (Wirtz et al., 2015).

In sterile inflammation processes during atherogenesis there are also neutrophils involved, notwithstanding the monocyte dominant role in atherosclerosis. Neutrophils mutual interaction with platelets based on both soluble mediators (especially cathepsin G/elastase and CCL5/CXCL4) and direct intercellular interactions causes increased neutrophil activation and recruitment at sites of endothelial damage. Increased monocyte activation, endothelial activation and myocardial infarction after plaque rupture are a result of enhancement of neutrophil effector functions, such as ROS production, granule secretion and neutrophil extracellular traps (NETs) formation, which is also a promising therapeutic target (Vajen et al., 2018).

Platelets as a therapeutic target

Atherothrombosis and secondary prevention

Platelets directly interact with endothelial cells and extracellular matrix proteins, secreting soluble mediators and recruiting additional platelets, thus becoming the main cellular contributors in primary hemostasis. When the endothelial monolayer is disrupted in the event of vascular damage or pathological conditions in atherosclerosis such as rupture of plaque, it reveals the perivascular tissue thrombogenic environment, which provokes thrombosis. A transmembrane receptor GPIb on the platelet surface, which forms a with GPIX and GPV and binds to von-Willebrand-Factor (vWF), also mediates initial platelet binding to sites of endothelial lesions (Bergmeier and Hynes, 2012). Formation of firm adhesion to the extracellular matrix (ECM) collagen by integrin GPVI and α2β1 follows reversible binding by GPIb-IX-V, taking into account high shear rates resistance, and moreover, GPVI increases intracellular concentration of calcium and thus triggers platelets activation, which leads to a number of processes crucial for formation of thrombus. The release of prothrombotic molecules such as thromboxane, serotonin and ADP is allowed by secretion of platelet granules and prostanoids produced by cyclooxygenase 1 (COX-1), which also provokes further activation of platelets through paracrine and autocrine signaling by G-protein coupled receptors. Formation of a stable thrombus is induced by "Inside-Out"-activation, that converts platelet integrins from a low-affinity to a high-affinity state and promotes platelet interaction with each other through integrin fibrinogen and αIIbβIII and with the ECM via α2β1, α5β1 and α6β1 (Sang et al., 2021).

In the past, the targets for antiplatelet therapy in cardiovascular disease were COX-1/2 mediated thromboxane production, platelet ADP-receptor P2Y12 and integrin αIIbβIII. Clopidogrel, Cangrelor, Ticagrelor and Prasugrel counteract ADP signaling through P2Y12 and, to varying degrees, P2Y1, whereas aspirin irreversibly inhibits platelet thromboxane production and COX-1/2, additionally, Tirofiban, Abciximab and Eptifibatid bind to αIIbβIII and thereby inhibit aggregation of platelets (Mansour et al., 2020). We schematically depicted the main antiplatelt drugs and targets in Figure 2.

The outcome of a meta-analysis from 2002 was confirmed by the results of a recent meta-analysis of the Antithrombotic Trialists Collaboration (ATT), which involved 16 randomized controlled trials. This meta-analysis of aspirin therapy for secondary prevention demonstrated a reduction in overall mortality by 10% and a considerable reduction by 19% in serious vascular events (stroke, myocardial infarction or death from vascular cause) (Antithrombotic Trialists' (ATT) Collaboration et al., 2009).

The advantages of antiplatelet therapy in patients with a higher risk of cardiovascular disease outweigh the extra risk of events of major bleeding, and a long-term aspirin therapy or inhibitors of P2Y12 for secondary prevention as an alternative is recommended based on these results (Schreuder et al, 2020).

Anti-platelet therapy in primary prevention

The efficacy of antiplatelet drugs in primary prevention is a topic that has been debated in the literature. While these drugs are commonly used to prevent clotting and reduce the risk of cardiovascular events, there are differing opinions on their effectiveness in individuals without prior cardiovascular diseases. Here are some key controversies and debates surrounding this topic:

Benefits vs. Risks: One of the main debates is the balance between the potential benefits of antiplatelet drugs in preventing cardiovascular events and the associated risks, particularly bleeding. Some studies have shown a reduction in cardiovascular events, such as heart attacks and strokes, with the use of antiplatelet therapy. However, these benefits must be weighed against the increased risk of bleeding, which can be serious and even life-threatening. Finding the optimal balance between benefits and risks remains a challenge.

Heterogeneity of Patient Populations: Another controversy revolves around the heterogeneity of patient populations included in primary prevention studies. Different individuals have varying baseline risks of cardiovascular events, and the benefits of antiplatelet drugs may differ accordingly. Some argue that antiplatelet therapy might be more effective in higher-risk individuals, while the benefits may be limited or outweighed by risks in lower-risk individuals. Identifying the appropriate patient

populations that can truly benefit from antiplatelet therapy is an ongoing challenge.

Individualized Approach: There is increasing recognition of the importance of personalized or individualized medicine. This approach considers a patient's unique characteristics, such as age, comorbidities, and bleeding risk, in determining the appropriateness of antiplatelet therapy. It raises the question of whether a generalized approach to prescribing these drugs for primary prevention is the most effective strategy or if targeted interventions based on individual risk profiles would yield better outcomes.

Changing Landscape: The literature on antiplatelet therapy is an evolving field, with new evidence emerging over time. As new studies are conducted and more data becomes available, the efficacy of antiplatelet drugs in primary prevention may be subject to re-evaluation. Clinical practice guidelines may also be updated to reflect any emerging controversies or changes in recommendations based on new evidence.

It's important to note that these discussions and controversies in the literature underline the need for further research and ongoing evaluation of the efficacy and safety of antiplatelet drugs in primary prevention. Ultimately, the decision to prescribe antiplatelet therapy should be based on an individualized assessment of risks and benefits, taking into account the specific characteristics and preferences of each patient.

The role of antiplatelet drugs in secondary prevention is undeniable, but their role in primary prevention is more debatable. The outcome of the analysis by the ATT from 2009, demonstrating a reduction by 12% serious vascular events after aspirin treatment in primary prevention, was confirmed by a recent meta-analysis on aspirin treatment in primary prevention by Zheng et al. which involved 13 trials from 1988 to 2018 and demonstrated that aspirin use is associated with considerable reductions in cardiovascular events (Zheng and Roddick, 2019). All anti-platelet drugs increase the risk of bleeding, platelets being key players in hemostasis regulation. In the studies mentioned above, increased risk of bleeding most likely outweighed the impact on reducing of cardiovascular events, so aspirin therapy was not associated with reduced total mortality in primary prevention (Eikelboom et al., 2012; Lupoli et al., 2017).

New light was shed on the antiplatelet treatment role in primary prevention after several trials were published in 2018. In the ASCEND trial, which assessed the impact of low-dose aspirin on primary prevention in patients with diabetes mellitus, a reduction by 12% in serious vascular events was a result of aspirin treatment. It is important to point out that this effect was observed in patients who received all other well-proven cardioprotective treatments, such as antihypertensive drugs and statins. Although patients with diabetes initially have a higher risk of cardiovascular events, however, an increased risk of bleeding substantially balanced these advantages (Bowman et al., 2018).

In reverse, bleeding risk was increased and no considerable reduction in cardiovascular events by aspirin therapy was shown in two other trials on aspirin in primary prevention in the elderly patients and patients with mild cardiovascular risk. Of these two trials, the ARRIVE trial (Gaziano et al., 2018) failed to identify the role of aspirin primary prevention, and the ASPREE trial (Mahady et al., 2021) including the elderly was powered enough for its endpoint.

Numerous animal studies, on the contrary, showed that aspirin or clopidogrel therapy had an inhibitory effect on the onset and progression of atherosclerosis. These data support the antiplatelet drugs use in primary prevention of atherosclerosis.

This exhibits the perplexity of present approaches in anti-platelet treatments for prevention of cardiovascular death. The aspirin or other anti-platelet drug use for primary prevention faces the challenge of treating with them only the patients whose cardiovascular event risk outweighs the risk of bleeding caused by these drugs, and antiplatelet drugs therapy cannot be declared an overall benefit even in case of diabetes, which is an important risk factor. On the other hand, if the antiplatelet drugs impact on hemostasis could be reduced to a minimum, would these drugs still be effective in preventing atherosclerosis? We think this is a plausible idea worth investigating (Ostergaard et al., 2017).

Discussion

Platelets play a crucial role in various physiological and pathological processes, including cardiovascular disease, inflammation, and atherosclerosis. Understanding the normal functioning of platelets and their interactions with other cells and molecules is essential for developing effective therapeutic strategies. This article provides insights into platelet biology, their involvement in atherogenesis, thrombus formation, inflammation, and platelet-endothelium interactions.

The review highlights the complex activation process of platelets, starting from their quiescent discoid state to their adhesion and aggregation at the site of vascular injury. This process involves the release of bioactive molecules from platelet granules, the conformational change of integrin receptors, and the formation of stable platelet aggregates. These mechanisms are vital for hemostasis and the initial stages of tissue repair.

In the context of atherosclerosis, the review emphasizes the role of platelets in the early stages of lesion development and atherothrombotic complications. Platelets can attach to the endothelium even without endothelial denudation, contributing to the progression of atherosclerotic lesions. Platelets release microparticles and granule proteins, interact with modified low-density lipoprotein, and cross-talk with endothelial and immune cells, thereby promoting atherogenesis.

Furthermore, the article discusses the relationship between platelets and inflammation. Platelets possess inflammatory functions and exhibit increased activation in advanced cardiovascular disease. Platelet activation and platelet-mediated inflammation may contribute to lipid retention, leukocyte recruitment, and the formation of asymptomatic thrombi. The review suggests that targeting platelet-mediated inflammation could be a potential approach for atherosclerosis prevention. Antiplatelet therapies such as aspirin and clopidogrel have shown anti-inflammatory effects in animal models and clinical studies. These therapies reduce vascular inflammation and inflammatory marker levels, contributing to plaque stabilization. However, the increased risk of bleeding associated with antiplatelet drugs needs to be considered when targeting platelet-mediated inflammation.

The interactions between platelets and the endothelium are crucial in both physiological and pathological conditions. Under normal conditions, endothelial cells maintain an antithrombotic phenotype through the expression of specific molecules and the secretion of substances that suppress platelet activation. However, endothelial dysfunction, which occurs in atherosclerosis, leads to the loss of these control mechanisms. Platelet adhesion to the endothelium involves specific receptors and interactions, including tethering, rolling, and firm adhesion. Platelet-endothelium crosstalk involves ligand-receptor interactions, secreted factors, and platelet-derived microparticles. Platelets contribute to endothelial activation and promote the upregulation of adhesion molecules and proinflammatory chemokines/cytokines. Understanding these interactions could help in identifying therapeutic targets for inflammatory and atherosclerotic conditions.

In conclusion, this review provides valuable insights into the normal functioning of platelets and their involvement in cardiovascular disease, inflammation, and atherosclerosis. It highlights the complex processes of platelet activation, adhesion, and aggregation, as well as their interactions with endothelial cells and other immune cells. The findings suggest that targeting platelet-mediated inflammation could be a potential therapeutic approach for atherosclerosis prevention. However, the balance between anti-inflammatory effects and the risk of bleeding needs to be carefully considered in the development of antiplatelet therapies. Further research is warranted to elucidate the underlying mechanisms and explore new therapeutic strategies aimed at modulating platelet function in these diseases.

Conclusion

The role of platelets in the normal functioning of the body has been studied very well. As well as their involvement in the process of thrombosis. More and more studies provide us with new data on the role of platelets in various pathologies, among which conditions associated with inflammation occupy a special place. One such condition is atherosclerosis. And if the role of platelets in the rupture of atherosclerotic plaques and the consequences of this is obvious, then their role in the earlier stages of atherogenesis usually escapes the attention of researchers. In this regard, the role of antiplatelet drugs in secondary prevention is undeniable, but their role in primary prevention is more debatable. For example, the use of aspirin and clopidogrel has been associated with a reduction in cardiovascular risk in some cases. However, despite the association of platelets with atherogenesis, and in particular inflammation, their role as a target for therapy has yet to be clarified.

Author contribution

A.V.P. wrote, drafted; V.N.S., V.A.O., A.Y.P., and A.N.O. wrote, reviewed and edited the paper.

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