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Review article

The role of platelets in the development and progression of pulmonary arterial hypertension

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ABSTRACT

Pulmonary arterial hypertension is a multifactorial disease characterized by vasoconstriction, vascular remodeling, inflammation and thrombosis. Although an increasing number of research confirmed that pulmonary artery endothelial cells, pulmonary artery smooth muscle cells as well as platelets have a role in the pulmonary arterial hypertension pathogenesis, it is still unclear what integrates these factors. In this paper, we review the evidence that platelets through releasing a large variety of chemokines could actively impact the pulmonary arterial hypertension pathogenesis and development. A recent publication revealed that not only an excess of platelet derived cytokines, but also a deficiency may be associated with pulmonary arterial hypertension development and progression. Hence, a simple platelet blockade may not be a correct action to treat pulmonary arterial hypertension. Our review aims to analyse the interactions between the platelets and different types of cells involved in pulmonary arterial hypertension pathogenesis. This knowledge could help to find novel therapeutic options and improve prognosis in this devastating disease.

1. Introduction

Pulmonary arterial hypertension (PAH) is a progressive state characterized by proliferative changes in the pulmonary vasculature and subsequent increased pulmonary vascular resistance (PVR), which leads to right-sided heart failure and premature death [1].

The pathogenesis of PAH involves excess vasoconstriction, vascular remodelling, inflammation and *in situ* thrombosis [2–5]. An imbalance between apoptosis and proliferation within the intima, media, and adventitia plays a crucial role in the PAH progression [6]. These pathological mechanisms lead to PVR increase due to narrowing the lumen of small pulmonary arteries.

Platelets are small disc-shaped megakaryocyte cell fragments, which lifespan is about 5–9 days. Platelets store growth factors, cytokines, and vasoactive substances in granules that can be released in a regulated manner upon stimulation (Fig. 1). Alpha granules contain, among others, P-selectin, transforming growth factor β (TGF-β1), platelet-derived growth factor (PDGF), β-thromboglobulin, platelet factor 4, Regulated on Activation, Normal T-cell Expressed and Secreted (RANTES), tumor necrosis factor α (TNFα), interleukin 1α (IL-1α), stromal-derived factor (SDF-1), interleukin 1β (IL-1β), tumor necrosis factor-like weak inducer of apoptosis (TWEAK), fibrinogen and coagulation factors V and XIII [7]. Delta (dense) granules contain serotonin (5-HT), calcium, and ADP/ATP. The activation of platelets, apart from releasing granules, increases the surface expression of various adhesion molecules and receptor (e.g. selectin P, gp IIIa/IIb) as well as the production of thromboxane A2 (TXA2), which in turn activates other platelets and promotes vasoconstriction and local thrombosis (Table 1).

There is growing evidence that inflammation plays an important role in the pathogenesis of PAH. Recent studies have confirmed the role of inflammatory modulators e.g. kynurenine metabolites, interleukin-6 and IL-1β, in the development of PAH [8–10]. Another one of them is the soluble tumor necrosis factor-like weak inducer of apoptosis (sTWEAK), which belongs to the TNFα superfamily [7,11]. This cytokine is involved in numerous biological responses associated with tissue damage and repair, such as apoptosis, cell growth or angiogenesis [11]. One of the main sources of circulating cytokines e.g. sTWEAK or P-selectin are platelets [7,12]. Microparticles derived from platelets, inflammatory cells, and the endothelium are an increasingly well-recognized signal in a variety of cardiovascular diseases, including thromboembolic events or PAH [13,14].

In this review, we sum up the evidence that platelets are involved in the development of PAH. We describe the thrombotic mechanisms

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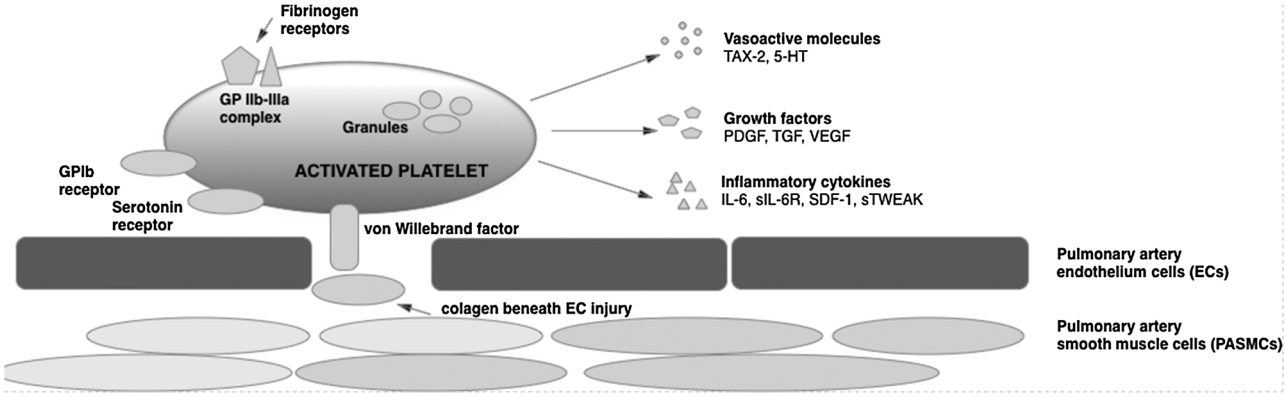

Fig. 1. At the site of pulmonary endothelium injury activated platelets attach to the vascular wall, start to aggregate and release e.g. inflammatory cytokines (e.g. IL-1, IL-6), vasoactive particles (5-HT, TXA-2) and growth (mitogenic) factors (PDGF, VEGF). This leads to pulmonary vasoconstriction, proliferation of smooth muscle cells and finally to pulmonary remodelling.
5-HT - 5-hydroxytryptamine; IL-1 - interleukin 1; IL-6 - interleukin 6; sIL-6R - soluble interleukin 6 receptor; SDF-1 - stromal derived factor 1; PDGF - platelet-derived growth factor; TGF - transforming growth factor; TXA-2 - thromboxane 2; VEGF - vascular endothelial growth factor.

Table 1
Major substances in pulmonary arterial hypertension pathophysiology released by activated platelets.

Group	Substance	Effect
Vasoactive substances	5-HT, Thromboxane A2	Increase vasoconstriction and impair the endothelial-smooth muscle cells (SMC) cross talk.
Mitogenic & growth factors	PDGF	Contributes to a higher proliferation rate of SMCs and fibroblasts.
	TGF-β1	Pulmonary smooth muscle cells over-proliferation leading to vascular remodelling.
	Insulin-like growth factor 1	
	VEGF	
	Epidermal growth factor	
	Thrombospondin 1	
Inflammatory cytokines	TNF-α, IL-1 α, IL-1β, IL-6	Exaggerate inflammatory response in ECs contributing to endothelial dysfunction.
	P-selectin	Promotes platelet aggregation and leukocyte migration to the injured site of endothelium.
	TWEAK	Modulates inflammatory processes and tissue healing.
	CXCL12 (SDF-1)	Localised in alpha granules, facilitates migration and differentiation of CD34 progenitor phenotype.
	sIL-6R	Binds to circulating IL-6 and associates with gp130, activating cells that express only gp130 on surface.

5-HT - 5-hydroxytryptamine; IL - interleukin; sIL-6R - soluble interleukin 6 receptor; SDF-1 - stromal derived factor 1; PDGF - platelet-derived growth factor; SDF - stromal derived factor; TGF - transforming growth factor; TNF - tumor necrosis factor; TWEAK - tumor necrosis factor-like weak inducer of apoptosis; VEGF - vascular endothelial growth factor.

through which platelets may be associated with this disease. The review is focused on molecules released by platelets during the inflammatory processes occurring in pulmonary arteries in PAH.

2. Review

2.1. Involvement of platelets in thrombotic processes in PAH

Thrombotic lesions are common pathological findings in PAH. Small vessel arteriopathy due to thromboembolic changes was found upon autopsy in 57% of PAH patients in a study from 1984 and for many years, thrombosis was considered a crucial factor in PAH pathogenesis [15]. The co-existence of pulmonary arteriopathy with recanalized thrombi in patients who did not have pulmonary thromboembolic disease cemented a role for thrombosis in the PAH pathogenesis. Some studies reported high frequencies (20%, 30%, and 56%) of thrombotic lesions in the histopathological classification of hypertensive pulmonary vascular disease [16–18]. Its role is still not fully elucidated. One theory was based on the fact that activation of coagulation contributes to the pathogenesis of PAH through luminal narrowing (both from the fibrin clot itself and related vascular remodelling, likely driven by proteases, tissue factor, factor Xa and thrombin). An alternate view is that thrombotic arteriopathy is only a bystander (epiphenomenon) of pulmonary vascular remodeling [19,20].

Tissue factor (TF) that together with von Willebrand Factor (vWF) during endothelial injury initiate coagulation cascade and platelet aggregation, could play an important role in thrombosis in situ occurring in PAH. In physiological conditions, TF is expressed at low levels in the pulmonary vessel wall, but it is increased in the vascular lesions in PAH patients [21]. PAH patients have also higher levels of circulating vWF, what is related to worse outcome [22]. Furthermore, endothelial cells (ECs) with higher expression of TF release more prothrombotic microparticles [23]. Enhanced platelet activation and coagulation cascade abnormalities (e.g. alterations of serotonin (5-HT), thromboxane (TXA2) or NO levels) overlap in PAH-related thrombosis [24].

Non-injured ECs by releasing nitric oxide (NO) and PGI2 - two important inhibitors of platelet aggregation - diminish thrombosis and through the synthesis and release of the profibrinolytic tissue plasminogen activator (t-PA) activate the fibrinolytic cascade. On the other hand, injured or activated ECs could produce the antifibrinolytic plasminogen activator inhibitor-1 (PAI-1) and enhance unfavourable prothrombotic processes [25,26]. TF exposure in injured ECs starts the coagulation process through complex formation with Factor VIIa, which in turn catalyses the activation of Factor X. They produce and release the vWF that attracts and activates platelets, as well as, thromboxane that contracts pulmonary smooth muscle cells (SMCs) and enhances platelets aggregation [19]. Platelets, by releasing various cytokines, affect SMCs and fibroblasts, simultaneously influencing inflammatory processes. Cytokines and chemokines recruit various inflammatory cells (T cells, B cells, macrophages, dendritic cells, mast cells) and these cells contribute to further release of chemokines, cytokines and growth factors, which in turn promote EC proliferation, migration and resistance to apoptosis, contributing to vascular remodeling and finally to arterial narrowing [6,14,27]. One of the chemokines potentially

affecting vascular remodelling - CXCL7 is stored in very high concentrations in platelets. CXCL7 enhances accumulation of neutrophils and activated mast cells in vascular tissues exaggerating inflammatory processes [28,29].

Thus, ECs and platelets are important regulators of the balance between prothrombotic and antithrombotic processes, which occur with changing intensity in many PAH patients.

There are attempts to create new PAH drugs targeting the mechanisms associated with thrombosis: anti-platelets drugs or anticoagulants. Examples of these drugs include thromboxane receptor antagonists [30] or thromboxane synthesis inhibitors, such as ozagrel and furegrelate. The first showed positive effects in a patient with portopulmonary hypertension and the second was tested in a hypoxia PAH experimental model, where pharmacological inhibition of TxA(2) synthase activity by furegrelate prevented the development of hypoxia-induced PAH by preserving the structural integrity of the pulmonary vasculature [31,32].

Despite the high prevalence of vascular thrombotic lesions at postmortem examinations of patients with PAH, according to ESC guidelines, the potential benefits of oral anticoagulants are still unclear and they may be considered only in patients with idiopathic or hereditary PAH and PAH secondary to anorexigens [1]. The usefulness of antiplatelet drugs in PAH therapy in the context of the latest reports is also questionable [12,27]. Not always the increased amount of cytokines stored in the platelets of PAH patients is associated with worse prognosis. Lowered sTWEAK platelet levels, involved in regional healing and angiogenesis processes, are linked with worse outcomes in PAH [12]. On the other hand, increased platelet content of SDF-1α and IL-6R was associated with worse prognosis [27]. Moreover, platelets play an active role in maintaining bioavailability of released microparticles that may also affect vascular remodelling in various ways. Further research and better understanding of altered platelet content and its release modulation are needed to fully elucidate the potential ways for new therapeutic targets. Therefore, simple blocking of platelets activation may not have beneficial influence on inflammatory processes in PAH [26].

It was also discussed that thrombocytopenia can be used as a prognostic indicator in patients with severe PAH, similarly as in other severe diseases [33–35]. There are a few theories about the mechanism of thrombocytopenia e.g. abnormal platelet distribution resulting from disorders of the spleen; ineffective thrombopoiesis or increased platelet destruction due to immunological processes. There is also a possibility that in PAH patients thrombocytopenia is caused by platelet consumption during thrombi formation in pulmonary bed [33]. Another report showed the elevation of mean platelet volume (MPV) and platelet distribution width (PDW) - two markers of platelet activation and increased turnover - in patients with idiopathic PAH, which correlated with hemodynamic parameters and partially with the disease severity [36]. Chronic thromboembolic pulmonary hypertension (CTEPH) is another type of pulmonary hypertension, in which thrombotic occlusion of pulmonary arteries, due to non-resolving but organizing thrombi, gradually leads to PVR increase. CTEPH is accompanied by a prothrombotic state, including platelet abnormalities (higher mean platelet volume) or impaired aggregation [37].

2.2. Platelets as a source of chemokines

Activated platelets are an important source of many inflammatory cytokines [38]. ECs physiologically inhibit platelet activation by producing nitric oxide and PGI2. In case of pulmonary vessel injury, the aggregation of platelets at the site of injury is enhanced and then various vasoactive and inflammatory factors are released [2,6].

One of the possible communication links between vascular cells and platelets in PAH is the activation of toll-like receptor 4 (TLR4) [39]. Lipopolysaccharide (LPS), a substance from bacterial cell wall, is a TLR4 agonist that enhances platelet P-selectin expression, IL-1β secretion and platelets aggregation in response to low-dose thrombin, making TLR a „bridge" between innate immunity and coagulation process [40]. Bauer et al. proved that platelet TLR4 deletion attenuated PAH development and RV hypertrophy in hypoxia-induced PAH models, suggesting that TLR4 may be a crucial link connecting pulmonary ECs and thrombocytes and that platelets are crucial for PAH development and progression [39].

Injury of ECs leads to local platelets activation and thrombus formation. As a consequence, platelets release various growth factors like PDGF or VEGF, inducing endothelial and smooth muscle cells proliferation. Although factors that mediate this initial injury are largely unknown, causes such as chronic hypoxia, viral infection, mechanical stretch or shear stress, can induce inflammatory processes [2,14].

Thrombocytes are the source of various small molecules that are stored inside platelet's granules. Dense granules contain molecules such as ATP, ADP, 5-HT and alpha granules contain platelet-factor 4 (PF4/CXCL4), TWEAK, IL-1α and IL-1β, TGF-β, TNF-α or PDGF [24]. Also, the presence of soluble IL-6 receptor in platelets was confirmed by Western-blot and ELISA techniques [41]. Furthermore, Marta et al. proved sIL-6R release during platelet activation induced by thrombin, ADP and epinephrine [41]. sIL-6R stored in platelets' granules and released to blood may amplify the local inflammatory reactions via IL-6 axis. Potent mitogen and chemoattractant for pulmonary artery smooth muscle cells (PASMCs) is PDGF (stored in alpha granules), the receptor expression of which is enhanced in the lungs of PAH patients [42]. Inhibition of PDGF actions by imatinib reversed pulmonary hypertension in the animal models of PAH, however IMPRES showed unsatisfactory response to the imatinib treatment [43]. Another platelet-derived vasoconstrictor and proinflammatory mediator elevated in PAH patients is thromboxane A2 (TXA2) [44].

Thus, thrombocytes activation in PAH does not only cause aggregation and thrombosis but also affects SMCs, ECs and extracellular matrix by actions of released granule content. Some of the molecules e.g. sTWEAK are noted as healing agents contributing to tissue regeneration and angiogenesis. Lower sTWEAK content in platelets was linked with worse prognosis in PAH, suggesting that simple blockade of platelet activation and diminishing release of all substances stored in platelets' granules may not be the correct strategy [12].

Thrombocytes are also an important source of SDF-1 and store this cytokine in their alpha granules. At the site of vascular injury SDF-1 increases recruitment of progenitor cells promoting healing/vascular regeneration mechanisms as well as modulates paracrine processes such as adhesion, proliferation or chemotaxis. SDF-1 mediates the migration and differentiation of progenitor cells into an endothelial phenotype to promote vascular repair or regeneration [45,46]. Taking these facts into consideration platelet-derived SDF-1 could be an important factor in maintaining the balance between local vascular regeneration and inflammation. Platelets are not only an important source of SDF-1 but are also subject to its regulation. SDF-1 released from platelets exerts an autocrine effect by binding to CXCR4 receptor localised on platelet's surface [45]. PAH patients are characterized by reduced bioavailable NO within the pulmonary circulation [47,48], which is a negative regulator of platelet activation. Importantly, decreased endothelial-derived prostacyclin and NO in PAH patients can potentiate for platelet activation [49]. Furthermore, prostacyclin synthase activity is diminished in the lungs of PAH subjects [50], and prostanoid replacement is the most-effective PAH therapy [1]. Prostacyclin, which activates cAMP mediated signalling, is able to inhibit platelet aggregation and it is tempting to consider platelet inhibition to be a part of the mechanism by which prostacyclins positively affect pulmonary circulation. Similarly, Hoeper et al. proved that riociguat can intensify the platelet inhibitory potential of prostanoids [51]. Thus, there is a growing evidence that the signaling imbalance in pulmonary vessels (excess thromboxane, insufficient NO and prostacyclin) may affect platelet activation in pulmonary bed.

2.3. Serotonin theory in PAH pathogenesis

Thrombosis *in situ* in PAH is a result of the pulmonary vascular microenvironment shift towards a procoagulant and antifibrinolytic pattern, what is reflected by increased plasma levels of platelet aggregating agents such as serotonin and thromboxane [52]. Aggregating platelets release a number of vasoactive substances that can evoke either vasoconstrictor or vasodilator responses.

For many years, tryptophan pathway was considered as a crucial target for novel therapeutic options. One of tryptophan metabolites is serotonin (5-HT, 5-hydroxytryptamine), a potent vasoconstrictor that affects neutrophil recruitment in inflammatory responses. The majority of peripheral serotonin is stored in platelets, which secrete it upon activation [53]. In 1960 s, an increased number of PAH cases was observed among obese women taking an anorectic drug (aminorex) that among other actions affects serotonin transporter (5-HTT) in the lungs, enhancing serotonin dependent vascular remodelling [54,55]. Similarly, in 1980 s many patients receiving another drug with similar mechanism – fenfluramine, were diagnosed with pulmonary hypertension. Interestingly, mice lacking tryptophan hydroxylase 1 (TPH1) (the enzyme mediating serotonin synthesis) display a 96% reduction in peripheral 5-HT, and protection from fenfluramine induced PAH [56,57]. Both compounds increased extracellular concentration of 5-HT in the lungs leading to excessive vasoconstriction and PAH development. In the next years, other research confirmed that not only anorectic drugs are linked with PAH incidence but the activation of entire tryptophan cascade could play an important role in the disease pathogenesis (including increased activity of tryptophan enzymes, serotonin transporters SERT and 5-HT receptors) [8,53].

Herve et al. observed that patients with PAH presented higher serum levels and lower platelet levels of serotonin than healthy controls. Serotonin released during in vitro platelet aggregation was higher in PAH patients than in controls suggesting that platelets could store 5-HT and actively participate in its bioavailability at the site of platelet activation [58].

Serotonin acts via serotonin receptors (5-HT1B, 5-HT2A and 5-HT2B) in the PASMCs, ECs and fibroblasts [59,60], stimulating proliferation processes leading to narrowing of pulmonary vessels. Despite the wealth of evidence linking 5-HT signaling to so many pathologic pathways and processes characteristic of PAH, the trials undertaken with 5-HT receptor inhibitors appeared discouraging. There were attempts to target serotonin transporter SERT. Registry for use of SERT inhibitors indicated a reduction in the risk of death in PAH patients, although these results did not reach statistical significance [61].

Elevated levels of circulating tryptophan were also reported in PAH patients [8]. Besides serotonin, major product of tryptophan pathway is kynurenine and its downstream metabolites. Recently, higher levels of kynurenine were linked with adverse clinical course in PAH patients. Furthermore, up-regulation of the kynurenine pathway was linked with observed dysregulation (Treg-Th17 imbalance) of immune system in PAH patients, suggesting that metabolic and inflammatory mechanisms could overlap and exaggerate pulmonary vascular remodeling in PAH [8].

3. Conclusions

Platelets affect many mechanisms associated with the pulmonary arterial hypertension e.g. thrombotic pulmonary vascular lesions, vasoconstriction and remodelling which are basic processes of pulmonary vascular pathology in PAH. It is not clear whether these abnormalities are primary and contribute to PAH development, or secondary to this disease. Platelets and molecules released by them are interesting and tempting therapeutic targets in this challenging disease with still high mortality.

Conflict of interest

The authors declare no conflict of interests.

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Authors contribution

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