

Platelets in Pulmonary Hypertension: a Causative Role or a Simple Association?

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Abstract

Pathophysiology of pulmonary arterial hypertension is based on three basic mechanisms: thrombotic pulmonary vascular lesions, vasoconstriction and vascular remodeling. Platelets are related to all of these mechanisms by their aggregation, production, storage and release of several mediators. The role of platelets is more prominent in some types of pulmonary arterial hypertension, including those which are secondary to inflammatory and infectious diseases, hemoglobinopathies, essential thrombocythemia, drugs, thromboembolism, and cardiac surgery. Most pulmonary antihypertensive drugs have a negative effect on platelets. In this review, the mechanisms of platelets association with pulmonary arterial hypertension, those types of pulmonary arterial hypertension with greatest platelet contribution to their pathophysiology, and the effects of pulmonary antihypertensive drugs on platelets are summarized.

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Key Words: Platelet; Pulmonary Hypertension; Pulmonary Vascular Resistance; Vasoconstriction

Introduction

Pulmonary hypertension (PH) is a disease characterized by increased pulmonary artery pressure (PAP) due to increased pulmonary vascular resistance (PVR) and/or large intra-cardiac or vascular left-to-right shunts (transfer of systemic pressures to the right side of the heart and pulmonary artery). PH increases right ventricular pressure and may lead to heart failure, disability and finally, death of the patient in most cases. PH can be idiopathic or secondary to other known diseases [1]. The newest classification of PH, known as Dana Point classification, categorized this disease into 5 main subclasses [2]. These include pulmonary arterial hypertension (PAH), PH owing to left heart diseases, PH owing to lung diseases and/or hypoxia, chronic thromboembolic

PH (CTEPH), and PH with unclear multifactorial mechanisms.

In 1958, Heath and Edward presented a pathological classification of PH into 6 progressive grades: I (retention of fetal type pulmonary vessels, II (medial hypertrophy with cellular intimal reaction), III (progressive fibrous vascular occlusion), IV (progressive generalized arterial dilatation with the formation of complex dilatation lesions; plexiform lesions), V (chronic dilation with formation of numerous dilation lesions and pulmonary hemosiderosis), and VI (necrotizing arteritis) [3].

Although PH is not generally regarded as an inflammatory disease, there are evidences that inflammation plays a great role in the pathogenesis of at least some of its types [4]. There is a complex association between platelets and

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this disease. There are sufficient evidences that platelets play a causative role in some situations, while a simple association seems to be the best explanation for the others. However, discrimination between a cause-and-effect role and a mere association is difficult for most cases.

Thrombotic pulmonary vascular lesions, vasoconstriction and remodeling are the basic mechanisms of pulmonary vascular pathology in PH [1]. Platelets are related to all of these mechanisms through different pathways. Platelet functional abnormalities, endothelial disintegrity or dysfunction, and impaired fibrinolysis/antithrombosis were found in idiopathic PH [5]. It is not clear whether these abnormalities are primary and contributory to PH development, or secondary to this disease.

This review tries to present the evidences about the relation of platelets to PH. It first describes the mechanisms through which platelets may be associated with this disease. Then, those types of PH in which platelets seem to have a greater association are reviewed. Last, the effects of pulmonary antihypertensive drugs on platelets are discussed.

Literature Search Strategy

MEDLINE was searched to find English papers published from January 2006 to June 2010 and review articles from January 2000 to the same date, using the combination of words "Platelet" and "Pulmonary Hypertension". The numbers of articles found were 213 and 114, respectively. Whenever the materials found via this basic research were unsatisfactory in providing information about a subitem of this review, older references were added through a more specific search. In addition, many other references were included based on the experts' opinions and citations found in the reviewed papers.

Mechanisms of platelets contribution to the development of PH

Platelet Aggregation: Platelets actively participate in clot formation. Pulmonary intravascular thrombosis and thrombotic arteriopathy are common pathological findings in

PH [1,3]. Increased thromboxane (TxA₂) and serotonin and decreased prostacyclin (PGI₂) and nitric oxide (NO) enhance platelet aggregation in PH patients [1].

Maeda et al found a subpopulation of PH patients with increased propensity to thrombosis as suggested by increased platelet protease-activated receptor 1 (PAR1, a key element in the activation of human platelets by thrombin) expression and PAR-mediated surface exposure of P-selectin (an adhesion molecule, a marker for in vivo platelet activation, and an essential component in thrombus formation), associated with thrombocytopenia [6]. Thrombocytopenia was seen in PH patients [7,8]. It is not clear whether thrombocytopenia in PH patients is an incidental finding, caused by platelet consumption in pulmonary vasculature, or resulted from platelet shearing due to pulmonary microangiopathy as suggested by Herve et al [9].

Interactions with Endothelial Cells[1]: Endothelial cells (EC) participate actively in the process of coagulation. They activate factor X, facilitate the formation of the thrombin-activating prothrombinase complex, activate the extrinsic pathway of coagulation by releasing tissue factor, and produce and release von Willebrand Factor (vWF). On the other hand, EC can inhibit thrombosis and potentiate fibrinolysis. They produce NO and PGI₂, potent inhibitors of platelet aggregation [10]. EC express thrombomodulin, a high affinity receptor for thrombin, on their surface which prevents cleavage of fibrinogen to fibrin. EC are a source of both tissue plasminogen activator (t-PA), an important activator of plasminogen in the fibrinolytic cascade and plasminogen activator inhibitor-1 (PAI-1), an inhibitor of t-PA. These facts show the importance of EC in regulating the fine balance of prothrombotic and antithrombotic processes.

There are evidences in favor of an imbalance in PH patients. In 19 out of 27 patients with idiopathic PAH, PAI-1 activity was elevated [11]. Welsh et al found coagulation abnormalities in both idiopathic PAH and secondary PH. Low soluble thrombomodulin, low fibrinolytic activity, and high fibrinolytic inhibitor levels were seen in idiopathic PH patients. Those with secondary PH had elevated fibrinogen levels, increased vWF, and a trend to increased t-PA [12].

It is noteworthy that an additional role for PAI-

1 in vascular remodeling was suggested [13]. The studies about such a role for PAI-1 in PH are limited in number and conflicting [13].

Eicosanoids: Activated platelets are the major producers of TxA_2 , a vasoconstrictive and proaggregatory eicosanoid. PGI_2 is synthesized by EC, including those in pulmonary circulation. It is a physiological antagonist of TxA_2 , inhibiting platelet aggregation and relaxing vascular smooth muscle. Abnormal production of eicosanoids has been demonstrated in idiopathic and secondary PH, including patients with congenital heart diseases before and after surgical correction of these diseases [14-19]. In children with left-to-right shunts and in adolescents with Eisenmenger syndrome, the ratio of TxA_2 to PGI_2 metabolites urinary excretion is elevated as compared to control subjects [14,15,19]. Prostacyclin analogues are important drugs in the treatment of PH, emphasizing the causative role of this eicosanoid in the development of PH. In contrary, it is not known whether TxA_2 changes are secondary or primary to PH [16].

Serotonin: Serotonin (5-hydroxytryptamine, 5HT) is produced in the central nervous system (CNS), enterochromaffin cells and limitedly in platelets. Although platelets are not a large producer of serotonin, they are a major storage site for this mediator outside the CNS. Platelets readily take up serotonin from plasma, leaving very little circulating [20]. Under certain circumstances, pulmonary EC can produce and secrete serotonin as well [21].

Serotonin may contribute to PH in several ways. First, it is a pulmonary vasoconstrictor, mainly through its $5\text{HT}_{1\text{B}}$ receptor. In mice and human, overexpression of $5\text{-HT}_{2\text{B}}$ (another serotonin receptor subtype) in the pulmonary arterial tree is associated with the development of PH [22].

Second, serotonin has mitogenic activity on pulmonary arterial smooth muscle cells (PASMC), causing their hypertrophy and proliferation [20,23,24]. It was speculated that highly selective serotonin transporter (SERT) plays an important role in this mechanism [20]. Serotonin interacts with this specific transporter to enter PASMC and inducing proliferation [19]. Patients with PH have PASMC with faster growth rate than normal subjects after stimulation by serotonin or serum. Selective serotonin transporter inhibitors fluoxetine and citalopram, but not serotonin

receptor antagonists ketanserin (a $5\text{HT}_{2\text{A}}$ antagonist) and GR127935 (a selective $5\text{HT}_{1\text{B}/1\text{D}}$ antagonist) can inhibit those effects [19,25]. SERT gene polymorphism was also found to be a determinant of PH severity [19,26]. In addition, a $5\text{HT}_{\text{A}2}$ -mediated p38 mitogen-activated protein kinase activation with mitogenic effects on pulmonary artery fibroblasts from chronically hypoxic rats was observed [19,27].

Last, serotonin stimulates platelet aggregation especially in combination with adenosine diphosphate and TxA_2 [20,28]. The significance of increased plasma levels of serotonin in PH is not clear. While some studies reported high plasma levels of serotonin in PH patients [5,29,30], some found normal values in their cohort [30,31]. Increased serotonin level may be secondary to its impaired metabolism, as pulmonary EC contribute greatly to its clearance and their damage can be a sign of idiopathic PH [5]. Successful therapy by a potent antiaggregatory agent, epoprostenol, and heart-lung transplantation did not lower plasma serotonin levels [5,29]. Breuer et al found higher urinary excretion of 5-hydroxyindolacetic acid (a major metabolite of serotonin) in PH patients with left to right shunts and contributed it to higher metabolism of serotonin in these patients [31].

von Willebrand Factor: Only EC and megakaryocytes synthesize this glycoprotein. vWF is present in the endothelial basement membrane and in plasma. In endothelial basement membrane, it serves to anchor platelets and EC to the matrix [32]. In plasma, vWF acts as a carrier for coagulation factor VIII, and within platelet granules as an adhesive protein. EC stimulation in pathological conditions, like PH is followed by a rapid release of vWF from storage granules into the circulation [32]. Plasma level of vWF is used as a marker for EC damage, and increased levels of vWF have been reported in patients with PH [33,34]. Plasma antigenic activity of vWF (vWF:Ag) can be a useful biochemical index for predicting a short-term prognosis in PH. A plasma vWF:Ag higher than 240% of standard activity was 54% sensitive and 93% specific for identifying patients who were unlikely to survive one year, with an overall predictive value of 75% [32]. Elevated baseline and follow up vWF levels were showed to be associated with worse survival [35].

Nitric Oxide: Endothelium-derived NO (eNO) is a potent inhibitor of platelet aggregation in addition

to its vasodilatory and antiproliferative effects. Deficiency in endothelial NO synthase (eNOS) sensitize mice to hypoxia-induced PH, whereas pulmonary gene transfer of eNOS could protect the lungs. Low NO levels were found in the exhaled breath of PH patients [36]. Tetrahydrobiopterin (BH4) is an essential cofactor for the enzymatic activity of all three isoenzymes of NOS, including eNOS [37]. Khoo et al showed that mice deficient in BH4 developed PH which can be reversed by increasing BH4 availability. They also found that augmented BH4 production can be protective against hypoxia-induced PH in mice [36].

Platelet Activating Factor: Platelet Activating Factor (PAF) is a phospholipid with diverse physiological and pathological actions [38]. Caplan et al reported high PAF plasma levels in newborns with persistent pulmonary hypertension of the newborn (PPHN), correlation between plasma PAF level and disease severity, and a fall in PAF levels as they improved clinically [39]. PAF has also been implicated in chronic hypoxia-induced PH in adult rats [40-42]. Bixby et al found increased PAF synthesis in pulmonary arteries, increased PAF receptor protein expression, increased PAF receptor binding, and an increase in PAF induced smooth muscle cell proliferation in fetal lambs exposed to chronic high altitude hypoxia [42].

Angiostatin: Angiostatin induces EC apoptosis. It is cleaved from plasminogen at the platelet plasma membrane, absorbed by platelet membrane, and released only upon aggregation [43-46]. Overexpression of angiostatin is associated with PH in mice [44,47]. Only upon thrombus formation in the pulmonary capillaries would excessive amounts of angiostatin be released in a localized manner, where it could contribute to the EC microfragment formation, injury and/or death, and possibly to the progression of PH [44]. Idiopathic PAH patients have significantly elevated platelet, but not plasma, angiostatin levels compared to controls [44].

Vascular Endothelial Growth Factor: Vascular endothelial growth factor (VEGF) is a growth factor involved in vasculogenesis and angiogenesis. Platelets contain large circulating stores of VEGF. This factor antagonizes the formation of apoptotic endothelial microfragments by angiostatin [44,48,49]. A possible role for VEGF in the development of PH is suggested but not approved yet [44]. VEGF

produced endogenously by EC is also crucial for the maintenance of vascular endothelium [50,51].

CD40 and its ligand: CD40 Ligand (CD40L) is a transmembrane protein found on the surface of CD4+ T cells and activated platelets as well as in plasma (soluble form) [52]. CD40 can be found on the B cells, macrophages, vascular smooth muscle and EC. CD40L may interact with CD40 ensuing inflammatory reactions, matrix degradation and thrombus formation [52]. Damas et al showed several evidences in favor of a role for this pathway in PH, including higher levels of soluble CD40L in secondary and idiopathic PH but not in CTEPH patients, lower levels in patients receiving warfarin, higher levels in arterial blood than in mixed venous samples (enhanced release or reduced clearance in pulmonary vasculature), and increased secretion of soluble CD40L by platelets of PH patients [52].

Platelet-Derived Growth Factor: Platelet-Derived Growth Factor (PDGF) is a disulphide-linked polypeptide comprised of two chains (A and B) and appearing as three dimeric isoforms termed PDGF AA, AB, and BB [53,54]. Many cell types including smooth muscle cells, and macrophages secrete PDGF-like molecules [53,54]. Existence of PDGF-AB in platelets may be confined to humans [54,55]. PDGF-B is processed in platelets into a soluble and active isoform lacking the retention motif [54]. A PDGF-like protein is secreted by EC [56]. Growth factors such as PDGF may participate in the initiation and/or progression of idiopathic PAH [57]. PDGF can induce the proliferation and migration of smooth cells and fibroblasts [57]. PDGF expression is elevated in lung biopsies of patients displaying idiopathic PAH [57]. There is an evidence that serotonin transactivates PDGF receptor (PDGFR) β through SERT in PASMOC [58].

LIGHT: Lymphotoxin-like Inducible protein that competes with Glycoprotein D for Herpesvirus entry mediator on T lymphocytes (LIGHT), is a platelet-derived member of the tissue necrosis factor superfamily [59]. Serum levels of LIGHT are increased in PH patients and its arterial level correlates with mortality. Immunostaining of LIGHT and its receptors was observed in alveolar macrophages, vascular smooth muscle cells, and EC in lungs from patients with PAH. In addition, prostacyclin therapy lowers the serum level of LIGHT. Based on these observations, it was suggested that LIGHT may have a role in the

pathogenesis of PH, although further studies were recommended as well [59].

Platelets and angiogenesis: Platelets seem to play a significant role in angiogenesis through their proangiogenic and antiangiogenic factors [60]. Platelets and megakaryocytes promote angiogenesis through proangiogenic factors like VEGF-A, fibroblast growth factor 2, epidermal growth factor, PDGF and matrix metalloproteinase 9 [48-61]. On the other hand, they inhibit angiogenesis by their antiangiogenic factors like platelet factor 4, thrombospondin 1, 2-macroglobulin, PAI-1, and angiostatin [48]. A disordered or misguided angiogenesis was suggested to be present in patients with severe PH [62]. The role of platelets in this situation is not clear.

Types of PH with greatest platelets contribution

Inflammatory and Connective Tissue Diseases: Inflammation appears to play a significant role in some types of PAH, including those secondary to connective tissue diseases [4]. PAH occurs in patients with systemic sclerosis (SS) and CREST syndrome (calcinosis, the Raynaud phenomenon, esophageal dysfunction, sclerodactyly, and telangiectasia), and less frequently in patients with systemic lupus erythematosus (SLE), rheumatoid arthritis, Takayasu arteritis, polymyositis, and dermatomyositis [63].

PAH is a major complication of SS [67]. It has always been among the leading causes of mortality in these patients [65]. Using echocardiography, it can be found in 13.3% of SS patients [66]. Survival of SS patients with PAH was reported to be 50% at 12 months [67]. It was hypothesized that the initial lesions appears due to vasoconstriction or vasospasm, but inflammation and excessive platelet adhesion lead to the vasculopathy [64]. Hummers et al found higher levels of PDGF and VEGF in patients with scleroderma [68]. Activation of the PDGF/PDFGR signaling pathway has been linked to some proliferative and fibrotic disorders including PAH and SS [69]. Baroni et al showed that autoantibodies against PDGF receptor are specific hallmark of SS [70]. It was suggested that these antibodies may contribute to the development of PAH in this disease [71]. SS is characterized by EC

injury as well [70].

PAH is also found in 0.5-14% of patients with SLE, being the third cause of death after infections and organ failure [72]. Platelet abnormalities are among the suggested mechanism for PAH development in SLE [72]. Some of these patients have experienced significant improvements with immunosuppressive therapy, emphasizing the importance of inflammation in this setting [4]. The development of PAH greatly deteriorates prognosis of these patients [72].

Antiphospholipid (aPL) antibodies have been detected in PAH due to thromboembolism and in the primary plexogenic PAH [73]. The prevalence of aPL antibodies in patients with CTEPH is 10-20% [74]. PAH may be the only manifestation of the antiphospholipid syndrome (APS), and patients have been observed with very high levels of aPL antibodies [73]. The prevalence of PAH in primary APS and secondary APS patients is around 3.5% and 1.8%, respectively [74]. PAH and aPL antibodies may also accompany SLE [72]. aPL antibodies found in a greater percentage of SLE patients with PAH than in those with normal PAP [72]. Interaction between aPL and EC and the resultant vascular remodeling was suggested as the basis of PAH development [72].

Human Immunodeficiency Virus (HIV) infection: Several cases of HIV-1 infected patients with idiopathic PAH have been reported, raising the question of a causal relationship between these two conditions [57,75-79]. The lack of evidence for a direct HIV-1 pulmonary artery infection by means of electron microscopy, immunochemistry, deoxyribonucleic acid in situ hybridization, and polymerase chain reaction in two patients displaying HIV-1-associated idiopathic PAH has suggested that HIV-1 may act in these cases through mediator release associated with retroviral infection rather than by direct endothelial infection [57,76].

Hemoglobinopathies: PAH is one of the complications of sickle cell disease (SCD). Mild PAH (PAP<35mmHg) is seen in 20% and moderate to severe degrees (PAP>45mmHg) in 10% of the patients [80]. Platelet activation may contribute to the development of PAH. Direct inhibition of NO by plasma hemoglobin released from damaged red blood cells leads to platelet activation because NO is a potent inhibitor of this pathway [80-82]. Clinical studies of patients with

SCD reveal correlations between the intrinsic rate of intravascular hemolysis and blood levels of procoagulant factors [80,83,84]. Cell-free plasma hemoglobin mediated resistance to NO and the development of PAH has also been shown in transgenic mouse models of SCD and spherocytosis, and in mouse models of alloimmune hemolysis and malaria [80,85,86].

NO bioavailability is decreased not only through NO scavenging by plasma hemoglobin and superoxide, but also through arginine depletion by plasma arginase, and increased NO inactivation by reactive oxygen species derived from xanthine oxidase, nicotinamide adenine dinucleotide phosphate-oxidase, hemoglobin S autooxidation, and uncoupled endothelial nitric oxide synthase (eNOS) [87]. In SCD patients, plasma level of PDGF BB correlated positively with tricuspid valve regurgitation velocity (which reflects systolic PAP), while that of VEGF negatively correlated [88]. Activated platelets might be a source of increased plasma PDGF levels in SCD [82,88].

PH is found in 10–75% of patients with thalassemia and can be the leading cause of heart failure in these patients [89-92]. Prior splenectomy, older age, and evidence for chronic hemolysis were significantly associated with PAH [92]. A suggested mechanism involves abnormal red cell membrane phosphatidylserine exposure. This can trigger low-grade hypercoagulability, which is enhanced in splenectomized patients [92-94]. Alternatively, there is a growing evidence that hemolysis-induced NO scavenging is responsible for the PAH development in thalassemia, causing platelet activation, thrombosis, and endothelial dysfunction [92,95]. Singer et al found higher soluble P-selectin levels in pulmonary hypertensive thalassemic patients in comparison with pulmonary normotensive ones. They also found lower protein C in these patients which may contribute to a hypercoagulable state, but its role in the development of PAH is unknown [92]. It was reported that a shorter platelets life span in both splenectomized and non-splenectomized patients with thalassemia than in non-thalassemic splenectomized patients [96,97].

Thromboembolic PH: Acute massive pulmonary thromboembolism (PTE) increases PAP and PVR [19]. In some subjects including a small number (0.1- 3.8%) of PTE patients, a chronic state of

thromboembolism leads to PH [98,99]. This different disease state is called CTEPH. The pathophysiology of CTEPH is not completely understood: proposed mechanisms include a t-PA/PAI-1 imbalance [100], and lysis-resistant fibrinogen variants [101,102]. A difficult situation is to distinguish an acute PH in an already normal PTE patient from an acute embolism in an unrecognized CTEPH patient as these two conditions need different treatment algorithms [103]. Lifelong anticoagulation is required for all CTEPH patients, while antiplatelet therapy is indicated in some [103].

PH Crisis after Cardiopulmonary Bypass: After cardiac surgery, a sudden increase in PAP and PVR may occur. The pathophysiology of these problematic and sometimes fatal episodes, called pulmonary hypertensive crisis [19], is complex and not well understood yet. The exposure of platelets to the artificial membrane during cardiopulmonary bypass may activate them and contribute to this process. Endothelial injury, hypothermia, and nonpulsatile perfusion may be more important factors than the artificial membrane in this situation [104]. The mechanism of endothelial injury is not well understood; however, ischemia-reperfusion injury could be its main basis. Endothelial injury impairs the balance between vasodilatory and vasoconstrictive mechanisms in favor of the latter, and promotes platelet aggregation and activation [104].

Drug- and toxin-induced PAH: PAH may develop after using anorexigen drugs such as fenfluramines, Aminorex and toxins like rapeseed oil [2]. Although there is an accepted belief that these drugs potentiate PAH through their serotonin releasing properties [22,105], there are controversies as dexfenfluramine can actually lower blood serotonin and may only slightly elevate plasma serotonin to nontoxic levels [105-107]. Often the fenfluramines and phentermine were co-administered. The combination of fenfluramine/phentermine is known to increase SERT activity [20,108].

Essential thrombocythemia (ET): Altintas et al found PH in 47.8% of ET patients. There was a statistically significant difference in platelet counts between ET patients with PH and without PH. In contrast, none of the patients with reactive thrombocytosis had PH [109].

PH therapy and platelets

Anticoagulation: Abnormalities of the activated clotting system [32,110-112], impaired fibrinolysis [11,112,113], abnormal platelet function [16,112,114], histological evidence of microvascular thrombosis [112,115,116], EC dysfunction and injury [1], and increased tendency to develop deep vein thrombosis due to low cardiac output and sedentary life in chronic patients are the rationale to use anticoagulants in PH [9,112]. However, there is no randomized controlled trial and only observational studies data supports their administration [9,112,117]. In a systematic review, Johnson et al reported that five observational studies suggested a survival benefit associated with warfarin in the treatment of idiopathic PH, whereas two others did not support this association [112]. Anticoagulants are indicated for idiopathic PH (class IIa, level of evidence C) and permitted for secondary PH (class IIb, level of evidence C) [117,118].

Prostacyclin and its analogues: Several reports have shown that prostacyclin therapy directly inhibits platelets [9,119-121]. Thrombocytopenia was observed in 34% of PH patients treated by epoprostenol [122]. The administered dose and severity of hemodynamic abnormalities were associated with thrombocytopenia. It is not clear whether epoprostenol, PH, or both cause this complication, although the drug seems to play a greater role. In one patient in whom epoprostenol was discontinued, platelet counts improved after discontinuation of epoprostenol despite worsening hemodynamics, and then fell after re-initiation at a lower dose despite improvement in hemodynamics, suggesting that epoprostenol caused the thrombocytopenia [122]. The mechanism of epoprostenol-induced thrombocytopenia is unknown, as well as its significance. In addition, prostacyclin therapy in PH was associated with a significant decrease in serum levels of LIGHT [59]. A recent study confirmed platelet inhibitory function of epoprostenol by preventing platelet aggregation and platelet-leukocyte conjugates formation [123]. This may suggest an additional mechanism for epoprostenol in the treatment of PH.

Aerosolized Iloprost was also showed to inhibit platelet aggregation which was mild but sustained [124]. The observed results on platelet

function were postulated to explain, at least partly, the beneficial effects of this drug in PH patients [124].

Phosphodiesterase Type 5 Inhibitors: NO is an important molecule in a number of cellular functions, including the regulation of vascular smooth muscle tone [125,126]. The physiological target of NO is soluble guanylate cyclase, the enzyme which catalyses the conversion of guanosine triphosphate (GTP) to the intracellular second messenger cyclic guanosine monophosphate (cGMP), mediating NO induced relaxation [126,127]. Intracellular cGMP is rapidly inactivated to guanosine monophosphate (GMP) by the action of cyclic nucleotide phosphodiesterases (PDEs). Therefore, cGMP concentration in smooth muscle cells is mainly dependent on the balance between the synthesis by soluble guanylate cyclase and the breakdown by PDEs, which represents the unique degradation pathway for this second messenger [126,128,129]. There exist 11 distinct PDE isoenzymes which differ in their substrates, stimulators, inhibitors or gene homology [126,130]. PDE5 is the isoenzyme highly presented in pulmonary vasculature [126,131,132], and pulmonary vasodilatation has been resulted from its inhibition [126,133-135].

Three commercially available PDE5 inhibitors are sildenafil, vardenafil and tadalafil. These inhibitors, specifically sildenafil, received attention for their action on the pulmonary vasculature and the observed beneficial effects in the treatment of PH [126,136-139]. As PDE5 is present in human platelets, the effect of NO and NO donors on platelet function is potentiated by sildenafil [82,140,141]. Enhancement of platelet sensitivity to NO by PDE5 inhibition returns platelet activation to more normal levels in patients with SCD and PAH [82]. This effect is added to the beneficial effects of sildenafil on endothelial function in these patients.

Vardenafil has independent calcium-channel blocking activity on platelets [126]. This drug significantly reduced the Ca²⁺ mobilization and Ca²⁺ influx in thrombin-stimulated rabbit washed platelets [126]. This action enhances pulmonary vasodilatory effect of Vardenafil.

Nitric Oxide: Inhaled NO is administered for the acute treatment of PH, mostly after cardiac surgery and in PPHN [19]. As mentioned previously, this drug is a potent inhibitor of platelet

activation. Beghetti et al found that 30 ppm inhaled NO inhibits platelet aggregation after stimulation by collagen, arachidonic acid, and epinephrine. Plasma but not intraplatelet cGMP levels were increased meanwhile. It was postulated that platelet effects of NO should be mediated through a cGMP-independent mechanism, in contrary to the vascular effects [142].

Endothelin Receptor Antagonists: Endothelin (ET) receptors were identified on human platelets [143]. ET-1 is overexpressed in PH and may be an important factor in the initiation or progression of the disease [144]. The ET-1 effects can be mediated directly through its receptors on the platelets, or indirectly through activation of prostaglandin synthesis [144,145]. Iannone et al showed that serum levels of the soluble form of platelet EC adhesion molecule-1 (PECAM-1) is increased in patients with systemic sclerosis and PAH, and bosentan therapy can return it to the normal values [146]. However, bosentan had no effect on serum vWF level in these patients [146].

Phosphodiesterase Type 3 Inhibitors: Pulmonary vasodilation can be induced by milrinone through its action on a cyclic adenylate monophosphate pathway [147]. The pulmonary antihypertensive action of this drug is well known [148]. Clinically, it is used for the treatment of PPHN and postoperative PH [149,150]. Thrombocytopenia is a major side effect of milrinone. Moreover, it has an inhibitory action on platelet activation [151].

It is not known whether the vasodilatory role of mirinone is solely responsible for its pulmonary antihypertensive action, or its antiplatelet role may be important as well. Kikura et al found no difference in platelet count, bleeding time, and platelet aggregation between cardiac post-operative patients receiving milrinone or not [152]. Tanaka et al speculated that at least part of the pulmonary antihypertensive action of milrinone may be through its platelet inhibitory properties [153].

PDGFR Inhibitors: The multi-kinase inhibitor (including PDGFR) imatinib mesylate reversed pulmonary vascular remodeling in rats with monocrotaline-induced PAH and in chronically hypoxic mice [154,155]. Addition of imatinib to approved PH drugs was reported to improve pulmonary hemodynamics and functional capacity of some patients with severe PAH [156-159]. A completed phase II clinical trial investigating the

safety and efficacy of imatinib mesylate in PH failed to meet the primary efficacy end point of improvement in exercise capacity. However, many secondary end points, including pulmonary hemodynamics, were significantly improved [159].

Sorafenib inhibits several kinases including PDGFR. It prevents pulmonary remodeling and improves cardiac and pulmonary function in experimental PH. The combined inhibition of tyrosine and serine/threonine kinases can provide an option to treat PH and associated right heart remodeling [160].

Serotonin antagonists: Although there is general agreement that serotonin is important in the pathophysiology of PAH, no serotonin antagonist exists with clinically accepted effects in this disease. Several agents are targets for investigation in human PAH, including the highly selective 5-HT_{2B} antagonist PRX-08066 and escitalopram (a serotonin reuptake inhibitor) [22].

There is a bulk of evidence in favor of a complex association between platelets and PH. Although there is a small number of reports against a causative role and a risk that many of these observations be secondary to PH [9], it seems that the role of these blood elements cannot be overlooked in the pathophysiology of PH. The author tried to provide a comprehensive collection of evidences; however, the presented list of associations between platelets and PH may be incomplete as these relations are numerous, complex, and many of them not well understood. In addition, PH is a heterogeneous disease with diverse pathophysiological bases, and the relation of platelets to each type may be different.

In spite of these evidences, the specific antiplatelet drugs such as aspirin and clopidogrel were not studied sufficiently in PH patients. In addition, no randomized clinical trial studied the role of anticoagulants in PH. These can make the basis of future researches on the relation of platelets and PH.

Conclusion

In summary, platelets not only participate actively in thrombus formation, but also produce (TxA₂, LIGHT, angiostatin, and PDGF), store (serotonin, vWF, and VEGF), and release mediators that may

contribute to the initiation or aggravation of PH (Fig. 1). Platelets are related to all three basic mechanisms of PH: vasoconstriction (serotonin and TxA₂), thrombotic lesions (aggregation, serotonin, TxA₂, CD40L, and vWF), and remodeling (serotonin, CD40L, proangiogenic and antiangiogenic factors).

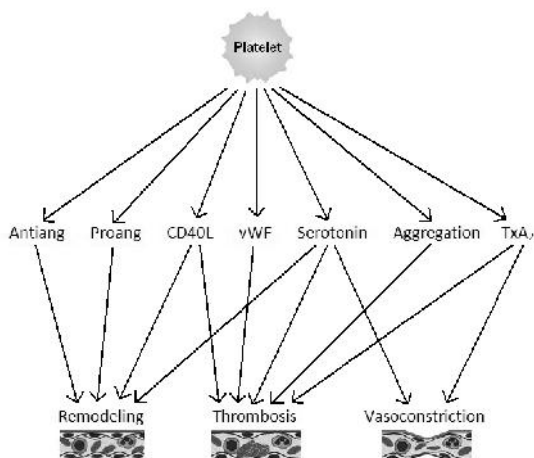


Fig 1: Summary of the major mechanisms of platelets associations with pulmonary vasoconstriction, thrombus formation, and remodeling in the pulmonary vessels. Antiang: antiangiogenic factors (platelet factor 4, thrombospondin 1, α_2 -macroglobulin, plasminogen activator inhibitor-1, and angiotatin); CD40L, CD40 ligand; Proang: proangiogenic factors (vascular endothelial growth factor A, fibroblast growth factor 2, epidermal growth factor, platelet-derived growth factor and matrix metalloproteinase 9); TxA₂: thromboxane A₂; vWF: von Willebrand Factor.

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Conflict of Interest: None

References

- Berger G, Azzam ZS, Hoffman R, Yigla M. Coagulation and anticoagulation in pulmonary arterial hypertension. *Isr Med Assoc J* 2009;11(6):376-9.
- Simonneau G, Robbins IM, Beghetti M, et al. Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol* 2009;54(1 Suppl):S43-54.
- Heath D, Edwards JE. The pathology of hypertensive pulmonary vascular disease; a description of six grades of structural changes in the pulmonary arteries with

special reference to congenital cardiac septal defects. *Circulation* 1958;18(4 Part 1):533-47.

- Dorfmüller P, Perros F, Balabanian K, et al. Inflammation in pulmonary arterial hypertension. *Eur Respir J* 2003;22(2):358-63.
- Kereveur A, Callebert J, Humbert M, et al. (2000) High plasma serotonin levels in primary pulmonary hypertension. Effect of long-term epoprostenol (prostacyclin) therapy. *Arterioscler Thromb Vasc Biol*;20(10):2233-9.
- Maeda NY, Carvalho JH, Otake AH, et al. Platelet protease-activated receptor 1 and membrane expression of P-selectin in pulmonary arterial hypertension. *Thromb Res* 2010;125(1):38-43.
- Stuard ID, Heusinkveld RS, Moss AJ. Microangiopathic hemolytic anemia and thrombocytopenia in primary pulmonary hypertension. *N Engl J Med* 1972;287(17):869-70.
- Suzuki H, Nakasato M, Sato S, et al. Microangiopathic hemolytic anemia and thrombocytopenia in a child with atrial septal defect and pulmonary hypertension. *Tohoku J Exp Med* 1997;181(3):379-84.
- Herve P, Humbert M, Sitbon O, et al. Pathobiology of pulmonary hypertension. The role of platelets and thrombosis. *Clin Chest Med* 2001;22(3):451-8.
- Riddell DR, Owen JS. Nitric oxide and platelet aggregation. *Vitam Horm* 1999;57:25-48.
- Eisenberg PR, Lucore C, Kaufman L, et al. Fibrinopeptide A levels indicative of pulmonary vascular thrombosis in patients with primary pulmonary hypertension. *Circulation* 1990;82(3):841-7.
- Welsh CH, Hassell KL, Badesch DB, et al. Coagulation and fibrinolytic profiles in patients with severe pulmonary hypertension. *Chest* 1996;110(3):710-7.
- Diebold I, Kraicun D, Bonello S, Gorlach A. The 'PAI-1 paradox' in vascular remodeling. *Thromb Haemost* 2008;100(6):984-91.
- Adatia I, Barrow SE, Stratton PD, et al. Thromboxane A₂ and prostacyclin biosynthesis in children and adolescents with pulmonary vascular disease. *Circulation* 1993 ;88(5 Pt 1):2117-22.
- Adatia I, Barrow SE, Stratton PD, et al. Effect of intracardiac repair on biosynthesis of thromboxane A₂ and prostacyclin in children with a left to right shunt. *Br Heart J* 1994;72(5):452-6.
- Christman BW, McPherson CD, Newman JH, et al. An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. *N Engl J Med* 1992;327(2):70-5.
- Fleming WH, Sarafian LB, Leuschen MP, et al. Serum concentrations of prostacyclin and thromboxane in children before, during, and after cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 1986;92(1):73-8.
- Greeley WJ, Bushman GA, Kong DL, et al. Effects of cardiopulmonary bypass on eicosanoid metabolism during pediatric cardiovascular surgery. *J Thorac Cardiovasc Surg* 1988;95(5):842-9.
- Beghetti M, Barst R, Naeije R, Rubin L. Pulmonary Arterial Hypertension Related to Congenital Heart Disease. Munich: Elsevier GmbH;2006.
- Mohammad-Zadeh LF, Moses L, Gwaltney-Brant SM. Serotonin: a review. *J Vet Pharmacol Ther* 2008;31(3):187-99.
- Sullivan CC, Du L, Chu D, et al. Induction of pulmonary hypertension by an angiotensin II/TIE2/serotonin

- pathway. *Proc Natl Acad Sci USA* 2003;100(21):12331-6.
22. O'Callaghan DS, Gaine SP. Combination therapy and new types of agents for pulmonary arterial hypertension. *Clin Chest Med* 2007;28(1):169-85.
 23. Ni W, Watts SW. 5-hydroxytryptamine in the cardiovascular system: focus on the serotonin transporter (SERT). *Clin Exp Pharmacol Physiol* 2006;33(7):575-83.
 24. Torres GE, Gainetdinov RR, Caron MG. Plasma membrane monoamine transporters: structure, regulation and function. *Nat Rev Neurosci* 2003;4(1):13-25.
 25. Marcos E, Fadel E, Sanchez O, et al. Serotonin-induced smooth muscle hyperplasia in various forms of human pulmonary hypertension. *Circ Res* 2004;94(9):1263-70.
 26. Eddahibi S, Morrell N, d'Ortho MP, et al. Pathobiology of pulmonary arterial hypertension. *Eur Respir J* 2002;20(6):1559-72.
 27. Welsh DJ, Harnett M, MacLean M, Peacock AJ. Proliferation and signaling in fibroblasts: role of 5-hydroxytryptamine_{2A} receptor and transporter. *Am J Respir Crit Care Med* 2004;170(3):252-9.
 28. Cerrito F, Lazzaro MP, Gaudio E, et al. 5HT₂-receptors and serotonin release: their role in human platelet aggregation. *Life Sci* 1993;53(3):209-15.
 29. Herve P, Launay JM, Scrobocaci ML, et al. Increased plasma serotonin in primary pulmonary hypertension. *Am J Med* 1995;99(3):249-54.
 30. Lederer DJ, Horn EM, Rosenzweig EB, et al. Plasma serotonin levels are normal in pulmonary arterial hypertension. *Pulm Pharmacol Ther* 2008;21(1):112-4.
 31. Breuer J, Georgaraki A, Sieverding L, et al. Increased turnover of serotonin in children with pulmonary hypertension secondary to congenital heart disease. *Pediatr Cardiol* 1996;17(4):214-9.
 32. Lopes AA, Maeda NY. Circulating von Willebrand factor antigen as a predictor of short-term prognosis in pulmonary hypertension. *Chest* 1998;114(5):1276-82.
 33. Collados MT, Sandoval J, Lopez S, et al. Characterization of von Willebrand factor in primary pulmonary hypertension. *Heart Vessels* 1999;14(5):246-52.
 34. Lip GY, Blann A. von Willebrand factor: a marker of endothelial dysfunction in vascular disorders? *Cardiovasc Res* 1997;34(2):255-65.
 35. Kawut SM, Horn EM, Berekashvili KK, et al. von Willebrand factor independently predicts long-term survival in patients with pulmonary arterial hypertension. *Chest* 2005;128(4):2355-62.
 36. Khoo JP, Zhao L, Alp NJ, et al. Pivotal role for endothelial tetrahydrobiopterin in pulmonary hypertension. *Circulation* 2005;111(16):2126-33.
 37. Katusic ZS, d'Uscio LV, Nath KA. Vascular protection by tetrahydrobiopterin: progress and therapeutic prospects. *Trends Pharmacol Sci* 2009;30(1):48-54.
 38. Ishii S, Shimizu T. Platelet-activating factor (PAF) receptor and genetically engineered PAF receptor mutant mice. *Prog Lipid Res* 2000;39(1):41-82.
 39. Caplan MS, Hsueh W, Sun XM, et al. Circulating plasma platelet activating factor in persistent pulmonary hypertension of the newborn. *Am Rev Respir Dis* 1990;142(6 Pt 1):1258-62.
 40. Chen D, Chen W. Changes of distribution of platelet activating factor in the lung of rats with hypoxic pulmonary hypertension. *Chin Med J (Engl)* 1996;109(10):776-9.
 41. Ono S, Westcott JY, Voelkel NF. PAF antagonists inhibit pulmonary vascular remodeling induced by hypobaric hypoxia in rats. *J Appl Physiol* 1992;73(3):1084-92.
 42. Bixby CE, Ibe BO, Abdallah MF, et al. Role of platelet-activating factor in pulmonary vascular remodeling associated with chronic high altitude hypoxia in ovine fetal lambs. *Am J Physiol Lung Cell Mol Physiol* 2007;293(6):L1475-82.
 43. Jurasz P, Alonso D, Castro-Blanco S, et al. Generation and role of angiostatin in human platelets. *Blood* 2003;102(9):3217-23.
 44. Jurasz P, Ng D, Granton JT, et al. Elevated platelet angiostatin and circulating endothelial microfragments in idiopathic pulmonary arterial hypertension: A preliminary study. *Thromb Res* 2010;125(1):53-60.
 45. Jurasz P, Santos-Martinez MJ, Radomska A, Radomski MW. Generation of platelet angiostatin mediated by urokinase plasminogen activator: effects on angiogenesis. *J Thromb Haemost* 2006;4(5):1095-106.
 46. Lucas R, Holmgren L, Garcia I, et al. Multiple forms of angiostatin induce apoptosis in endothelial cells. *Blood* 1998;92(12):4730-41.
 47. Pascaud MA, Griscelli F, Raoul W, et al. Lung overexpression of angiostatin aggravates pulmonary hypertension in chronically hypoxic mice. *Am J Respir Cell Mol Biol* 2003;29(4):449-57.
 48. Italiano JE, Jr., Richardson JL, Patel-Hett S, et al. Angiogenesis is regulated by a novel mechanism: pro- and antiangiogenic proteins are organized into separate platelet alpha granules and differentially released. *Blood* 2008;111(3):1227-33.
 49. Salven P, Orpana A, Joensuu H. Leukocytes and platelets of patients with cancer contain high levels of vascular endothelial growth factor. *Clin Cancer Res* 1999;5(3):487-91.
 50. Helotera H, Alitalo K. The VEGF family, the inside story. *Cell* 2007;130(4):591-2.
 51. Lee S, Chen TT, Barber CL, et al. Autocrine VEGF signaling is required for vascular homeostasis. *Cell* 2007;130(4):691-703.
 52. Damas JK, Otterdal K, Yndestad A, et al. Soluble CD40 ligand in pulmonary arterial hypertension: possible pathogenic role of the interaction between platelets and endothelial cells. *Circulation* 2004;110(8):999-1005.
 53. Heldin CH. Structural and functional studies on platelet-derived growth factor. *EMBO J* 1992;11(12):4251-9.
 54. Andrae J, Gallini R, Betsholtz C. Role of platelet-derived growth factors in physiology and medicine. *Genes Dev* 2008;22(10):1276-312.
 55. Stroobant P, Waterfield MD. Purification and properties of porcine platelet-derived growth factor. *EMBO J* 1984;3(12):2963-7.
 56. DiCorleto PE, Bowen-Pope DF. Cultured endothelial cells produce a platelet-derived growth factor-like protein. *Proc Natl Acad Sci USA* 1983;80(7):1919-23.
 57. Humbert M, Monti G, Fartoukh M, et al. Platelet-derived growth factor expression in primary pulmonary hypertension: comparison of HIV seropositive and HIV seronegative patients. *Eur Respir J* 1998;11(3):554-9.
 58. Liu Y, Li M, Warburton RR, et al. The 5-HT transporter transactivates the PDGFbeta receptor in pulmonary artery smooth muscle cells. *FASEB J* 2007;21(11):2725-34.

59. Otterdal K, Andreassen AK, Yndestad A, et al. Raised LIGHT levels in pulmonary arterial hypertension: potential role in thrombus formation. *Am J Respir Crit Care Med* 2008;177(2):202-7.
60. Rhee JS, Black M, Schubert U, et al. The functional role of blood platelet components in angiogenesis. *Thromb Haemost* 2004;92(2):394-402.
61. Kopp HG, Hooper AT, Broekman MJ, et al. cells determine angiogenic switch and extent of revascularization. *J Clin Invest* 2006;116(12):3277-91.
62. Tudor RM, Voelkel NF. Angiogenesis and pulmonary hypertension: a unique process in a unique disease. *Antioxid Redox Signal* 2002;4(5):833-43.
63. Rabinovitch M. Pathophysiology of Pulmonary Hypertension. In: Allen H, DJ. D, RE. S, TF. F (eds). Moss and Adams' Heart Disease in Infants, Children, and Adolescents. Philadelphia: Lippincott Williams & Wilkins; 2008.
64. Coral-Alvarado P, Quintana G, Garces MF, et al. Potential biomarkers for detecting pulmonary arterial hypertension in patients with systemic sclerosis. *Rheumatol Int* 2009;29(9):1017-24.
65. Steen VD, Medsger TA. Changes in causes of death in systemic sclerosis, 1972-2002. *Ann Rheum Dis* 2007;66(7):940-4.
66. Wigley FM, Lima JA, Mayes M, et al. () The prevalence of undiagnosed pulmonary arterial hypertension in subjects with connective tissue disease at the secondary health care level of community-based rheumatologists (the UNCOVER study). *Arthritis Rheum* 2005;52(7):2125-32.
67. Denton CP, Black CM. Pulmonary hypertension in systemic sclerosis. *Rheum Dis Clin North Am* 2003;29(2):335-49, vii.
68. Hummers LK, Hall A, Wigley FM, Simons M. Abnormalities in the regulators of angiogenesis in patients with scleroderma. *J Rheumatol* 2009;36(3):576-82.
69. Trojanowska M. Role of PDGF in fibrotic diseases and systemic sclerosis. *Rheumatology (Oxford)*:2008;47 (suppl 5):v2-4.
70. Baroni SS, Santillo M, Bevilacqua F, et al. Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. *N Engl J Med* 2006;354(25):2667-76.
71. Okamoto H. Stimulatory autoantibodies to the PDGF receptor in scleroderma. *N Engl J Med* 2006;355(12):1278;author reply 9.
72. Cefle A, Inanc M, Sayarlioglu M, et al. Pulmonary hypertension in systemic lupus erythematosus: relationship with antiphospholipid antibodies and severe disease outcome. *Rheumatol Int* 2011;31(2):183-9.
73. Asherson RA, Cervera R. Pulmonary hypertension, antiphospholipid antibodies, and syndromes. *Clin Rev Allergy Immunol* 2007;32(2):153-8.
74. Stojanovich L. Pulmonary manifestations in antiphospholipid syndrome. *Autoimmun Rev* 2006;5(5):344-8.
75. Coplan NL, Shimony RY, Ioachim HL, et al. Primary pulmonary hypertension associated with human immunodeficiency viral infection. *Am J Med* 1990;89(1):96-9.
76. Mette SA, Palevsky HI, Pietra GG, et al. Primary pulmonary hypertension in association with human immunodeficiency virus infection. A possible viral etiology for some forms of hypertensive pulmonary arteriopathy. *Am Rev Respir Dis* 1992;145(5):1196-200.
77. Opravil M, Pechere M, Speich R, et al. HIV-associated primary pulmonary hypertension. A case control study. Swiss HIV Cohort Study. *Am J Respir Crit Care Med* 1997;155(3):990-5.
78. Petitpretz P, Brenot F, Azarian R, et al. Pulmonary hypertension in patients with human immunodeficiency virus infection. Comparison with primary pulmonary hypertension. *Circulation* 1994;89(6):2722-7.
79. Polos PG, Wolfe D, Harley RA, et al. Pulmonary hypertension and human immunodeficiency virus infection. Two reports and a review of the literature. *Chest* 1992;101(2):474-8.
80. Kato GJ, Hebbel RP, Steinberg MH, et al. Vasculopathy in sickle cell disease: Biology, pathophysiology, genetics, translational medicine, and new research directions. *Am J Hematol* 2009;84(9):618-25.
81. Raghavachari N, Xu X, Harris A, et al. Amplified expression profiling of platelet transcriptome reveals changes in arginine metabolic pathways in patients with sickle cell disease. *Circulation* 2007;115(12):1551-62.
82. Villagra J, Shiva S, Hunter LA, et al. Platelet activation in patients with sickle disease, hemolysis-associated pulmonary hypertension, and nitric oxide scavenging by cell-free hemoglobin. *Blood* 2007;110(6):2166-72.
83. Ataga KI, Moore CG, Hillery CA, et al. Coagulation activation and inflammation in sickle cell disease-associated pulmonary hypertension. *Haematologica* 2008;93(1):20-6.
84. van Beers EJ, Spronk HM, Ten Cate H, et al. No association of the hypercoagulable state with sickle cell disease related pulmonary hypertension. *Haematologica* 2008;93(5):e42-4.
85. Gramaglia I, Sobolewski P, Meays D, et al. Low nitric oxide bioavailability contributes to the genesis of experimental cerebral malaria. *Nat Med* 2006;12(12):1417-22.
86. Hsu LL, Champion HC, Campbell-Lee SA, et al. Hypertension due to global impairment in nitric oxide bioavailability. *Blood* 2007;109(7):3088-98.
87. Wood KC, Hsu LL, Gladwin MT. Sickle cell disease vasculopathy: a state of nitric oxide resistance. *Free Radic Biol Med* 2008;44(8):1506-28.
88. Niu X, Nouraie M, Campbell A, et al. Angiogenic and inflammatory markers of cardiopulmonary changes in children and adolescents with sickle cell disease. *PLoS One* 2009;4(11):e7956.
89. Aessopos A, Farmakis D, Karagiorga M, et al. Cardiac involvement in thalassemia intermedia: a multicenter study. *Blood* 2001;97(11):3411-6.
90. Du ZD, Roguin N, Milgram E, et al. Pulmonary hypertension in patients with thalassemia major. *Am Heart J* 1997;134(3):532-7.
91. Jootar P, Fucharoen S. Cardiac involvement in beta-thalassemia/hemoglobin E disease: clinical and hemodynamic findings. *Southeast Asian J Trop Med Public Health* 1990;21(2):269-73.
92. Singer ST, Kuypers FA, Styles L, et al. association with platelet activation and hypercoagulable state. *Am J Hematol* 2006;81(9):670-5.
93. Borenstain-Ben Yashar V, Barenholz Y, Hy-Am E, et al. Phosphatidylserine in the outer leaflet of red blood cells from beta-thalassemia patients may explain the chronic

- hypercoagulable state and thrombotic episodes. *Am J Hematol* 1993;44(1):63-5.
94. Manodori AB, Barabino GA, Lubin BH, Kuypers FA. Adherence of phosphatidylserine-exposing erythrocytes to endothelial matrix thrombospondin. *Blood* 2000;95(4):1293-300.
 95. Rother RP, Bell L, Hillmen P, Gladwin MT. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. *JAMA* 2005;293(13):1653-62.
 96. Eldor A, Rachmilewitz EA. The hypercoagulable state in thalassemia. *Blood* 2002;99(1):36-43.
 97. Phrommintikul A, Sukonthasarn A, Kanjanavanit R, Nawarawong W. Splenectomy: a strong risk factor for pulmonary hypertension in patients with thalassaemia. *Heart* 2006;92(10):1467-72.
 98. Auger WR, Fedullo PF (2009) Chronic thromboembolic pulmonary hypertension. *Semin Respir Crit Care Med*;30(4):471-83.
 99. Lang IM, Klepetko W. Chronic thromboembolic pulmonary hypertension: an updated review. *Curr Opin Cardiol* 2008;23(6):555-9.
 100. Lang IM, Marsh JJ, Olman MA, et al. Parallel analysis of tissue-type plasminogen activator and type 1 plasminogen activator inhibitor in plasma and endothelial cells derived from patients with chronic pulmonary thromboemboli. *Circulation* 1994;90(2):706-12.
 101. Morris TA, Marsh JJ, Chiles PG, et al. Abnormally sialylated fibrinogen gamma-chains in a patient with chronic thromboembolic pulmonary hypertension. *Thromb Res* 2007;119(2):257-9.
 102. Morris TA, Marsh JJ, Chiles PG, et al. Fibrin derived from patients with chronic thromboembolic pulmonary hypertension is resistant to lysis. *Am J Respir Crit Care Med* 2006;173(11):1270-5.
 103. McNeil K, Dunning J. Chronic thromboembolic pulmonary hypertension (CTEPH). *Heart* 2007;93(9):v1152-8.
 104. Riedel B. The pathophysiology and management of perioperative pulmonary hypertension with specific emphasis on the period following cardiac surgery. *Int Anesthesiol Clin* 1999;37(2):55-79.
 105. MacLean MR, Dempsey Y. Serotonin and pulmonary hypertension--from bench to bedside? *Curr Opin Pharmacol* 2009;9(3):281-6.
 106. Martin F, Artigas F. Simultaneous effects of p-chloroamphetamine, d-fenfluramine, and reserpine on free and stored 5-hydroxy-tryptamine in brain and blood. *J Neurochem* 1992;59(3):1138-44.
 107. Zolkowska D, Baumann MH, Rothman RB. Chronic fenfluramine administration increases plasma serotonin (5-hydroxytryptamine) to nontoxic levels. *J Pharmacol Exp Ther* 2008;324(2):791-7.
 108. Belohlavkova S, Simak J, Kokesova A, et al. Fenfluramine-induced pulmonary vasoconstriction: role of serotonin receptors and potassium channels. *J Appl Physiol* 2001;91(2):755-61.
 109. Altintas A, Karahan Z, Pasa S, et al. Essential thrombocythemia and reactive thrombocytosis. *Leuk Lymphoma* 2007;48(10):1981-7.
 110. Geggel RL, Carvalho AC, Hoyer LW, Reid LM. von Willebrand factor abnormalities in primary pulmonary hypertension. *Am Rev Respir Dis* 1987;135(2):294-9.
 111. Lopes AA, Maeda NY, Aiello VD, et al. Abnormal multimeric and oligomeric composition is associated with enhanced endothelial expression of von Willebrand factor in pulmonary hypertension. *Chest* 1993;104(5):1455-60.
 112. Johnson SR, Mehta S, Granton JT. Anticoagulation in pulmonary arterial hypertension: a qualitative systematic review. *Eur Respir J* 2006;28(5):999-1004.
 113. Langleben D, Moroz LA, McGregor M, Lisbona R. Decreased half-life of fibrinogen in primary pulmonary hypertension. *Thromb Res* 1985;40(4):577-80.
 114. Kanai Y, Hori S, Tanaka T, et al. Role of 5-hydroxytryptamine in the progression of monocrotaline induced pulmonary hypertension in rats. *Cardiovasc Res* 1993;27(9):1619-23.
 115. Bjornsson J, Edwards WD. Primary pulmonary hypertension: a histopathologic study of 80 cases. *Mayo Clin Proc* 1985;60(1):16-25.
 116. Wagenvoort CA. Lung biopsy specimens in the evaluation of pulmonary vascular disease. *Chest* 1980;77(5):614-25.
 117. Galie N, Torbicki A, Barst R, et al. Guidelines on diagnosis and treatment of pulmonary arterial hypertension. The Task Force on Diagnosis and Treatment of Pulmonary Arterial Hypertension of the European Society of Cardiology. *Eur Heart J* 2004;25(24):2243-78.
 118. Bryniarski L, Pelc-Nowicka A, Zabojszcz M, Mirek-Bryniarska E. Dual antiplatelet therapy and antithrombotic treatment: Recommendations and controversies. *Cardiol J* 2009;16(2):179-89.
 119. Barst RJ, Rubin LJ, Long WA, et al. A comparison of continuous intravenous epoprostenol (prostacyclin) with conventional therapy for primary pulmonary hypertension. The Primary Pulmonary Hypertension Study Group. *N Engl J Med* 1996;334(5):296-302.
 120. Sakamaki F, Kyotani S, Nagaya N, et al. Increased plasma P-selectin and decreased thrombomodulin in pulmonary arterial hypertension were improved by continuous prostacyclin therapy. *Circulation* 2000;102(22):2720-5.
 121. Saleh JA. Role of iloprost and bosentan in pulmonary arterial hypertension. *Niger J Med* 2008;17(1):13-9.
 122. Chin KM, Channick RN, de Lemos JA, et al. Hemodynamics and epoprostenol use are associated with thrombocytopenia in pulmonary arterial hypertension. *Chest* 2009;135(1):130-6.
 123. Tamburrelli C, Crescente M, Izzi B, et al. Epoprostenol inhibits human platelet-leukocyte mixed conjugate and platelet microparticle formation in whole blood. *Thromb Res* 2011;128(5):446-51.
 124. Beghetti M, Reber G, de MP, et al. Aerosolized iloprost induces a mild but sustained inhibition of platelet aggregation. *Eur Respir J* 2002;19(3):518-24.
 125. Furchgott RF, Vanhoutte PM. Endothelium-derived relaxing and contracting factors. *FASEB J* 1989;3(9):2007-18.
 126. Toque HA, Teixeira CE, Priviero FB, et al. Vardenafil, but not sildenafil or tadalafil, has calcium-channel blocking activity in rabbit isolated pulmonary artery and human washed platelets. *Br J Pharmacol* 2008;154(4):787-96.
 127. Murad F. Role of cyclic GMP in the mechanism of action of nitrovasodilators, endothelium-dependent agents and atrial natriuretic peptide. *Biochem Soc Trans* 1988;16(4):490-2.

128. Maurice DH, Palmer D, Tilley DG, et al. Cyclic nucleotide phosphodiesterase activity, expression, and targeting in cells of the cardiovascular system. *Mol Pharmacol* 2003;64(3):533-46.
129. Rybalkin SD, Yan C, Bornfeldt KE, Beavo JA. Cyclic GMP phosphodiesterases and regulation of smooth muscle function. *Circ Res* 2003;93(4):280-91.
130. Bender AT, Beavo JA. Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol Rev* 2006;58(3):488-520.
131. Giordano D, De Stefano ME, Citro G, et al. Expression of cGMP-binding cGMP-specific phosphodiesterase (PDE5) in mouse tissues and cell lines using an antibody against the enzyme amino-terminal domain. *Biochim Biophys Acta* 2001;1539(1-2):16-27.
132. Hanson KA, Burns F, Rybalkin SD, et al. Developmental changes in lung cGMP phosphodiesterase-5 activity, protein, and message. *Am J Respir Crit Care Med* 1998;158(1):279-88.
133. Thusu KG, Morin FC, 3rd, Russell JA, Steinhorn RH. The cGMP phosphodiesterase inhibitor zaprinast enhances the effect of nitric oxide. *Am J Respir Crit Care Med* 1995;152(5 Pt 1):1605-10.
134. Tsai BM, Wang M, Pitcher JM, et al. Endothelium-dependent pulmonary artery vasorelaxation is dysfunctional in males but not females after acute lung injury. *Surgery* 2005;138(1):78-84.
135. Ziegler JW, Ivy DD, Wiggins JW, et al. Effects of dipyridamole and inhaled nitric oxide in pediatric patients with pulmonary hypertension. *Am J Respir Crit Care Med* 1998;158(5 Pt 1):1388-95.
136. Ghofrani HA, Grimminger F. Treatment of pulmonary arterial hypertension: phosphodiesterase-5 inhibitors. *Dtsch Med Wochenschr* 2006;131(49 Suppl 9):S311-4.
137. Ghofrani HA, Olschewski H, Seeger W, Grimminger F. Sildenafil for treatment of severe pulmonary hypertension and commencing right-heart failure. *Pneumologie* 2002;56(11):665-72.
138. Ghofrani HA, Wiedemann R, Rose F, et al. Sildenafil for treatment of lung fibrosis and pulmonary hypertension: a randomised controlled trial. *Lancet* 2002;360(9337):895-900.
139. Wilkens H, Guth A, König J, et al. Effect of inhaled iloprost plus oral sildenafil in patients with primary pulmonary hypertension. *Circulation* 2001;104(11):1218-22.
140. Gudmundsdottir IJ, McRobbie SJ, Robinson SD, et al. Sildenafil potentiates nitric oxide mediated inhibition of human platelet aggregation. *Biochem Biophys Res Commun* 2005;337(1):382-5.
141. Wallis RM, Corbin JD, Francis SH, Ellis P. Tissue distribution of phosphodiesterase families and the effects of sildenafil on tissue cyclic nucleotides, platelet function, and the contractile responses of trabeculae carneae and aortic rings in vitro. *Am J Cardiol* 1999;83(5A):3C-12C.
142. Beghetti M, Sparling C, Cox PN, et al. Inhaled NO inhibits platelet aggregation and elevates plasma but not intraplatelet cGMP in healthy human volunteers. *Am J Physiol Heart Circ Physiol* 2003;285(2):H637-42.
143. Touyz RM, Lariviere R, Schiffrin EL. Endothelin influences pHi of human platelets through protein kinase C mediated pathways. *Thromb Res* 1995;78(1):55-65.
144. Jagroop IA, Daskalopoulou SS, Mikhailidis DP. Endothelin-1 and human platelets. *Curr Vasc Pharmacol* 2005;3(4):393-9.
145. Yousufzai SY, Abdel-atif AA. Endothelin-1 stimulates the release of arachidonic acid and prostaglandins in cultured human ciliary muscle cells: activation of phospholipase A2. *Exp Eye Res* 1997;65(1):73-81.
146. Iannone F, Riccardi MT, Guiducci S, et al. Bosentan regulates the expression of adhesion molecules on circulating T cells and serum soluble adhesion molecules in systemic sclerosis-associated pulmonary arterial hypertension. *Ann Rheum Dis* 2008;67(8):1121-6.
147. Bassler D, Kreutzer K, McNamara P, et al. Milrinone for persistent pulmonary hypertension of the newborn. *Cochrane Database Syst Rev* 2010;11:CD007802.
148. Tanaka H, Tajimi K, Moritsune O, et al. Effects of milrinone on pulmonary vasculature in normal dogs and in dogs with pulmonary hypertension. *Crit Care Med* 1991;19(1):68-74.
149. Haraldsson SA, Kieler-Jensen N, Ricksten SE. The additive pulmonary vasodilatory effects of inhaled prostacyclin and inhaled milrinone in postcardiac surgical patients with pulmonary hypertension. *Anesth Analg* 2001;93(6):1439-45.
150. McNamara PJ, Laique F, Muang-In S, Whyte HE. Milrinone improves oxygenation in neonates with severe persistent pulmonary hypertension of the newborn. *J Crit Care* 2006;21(2):217-22.
151. Wesley MC, McGowan FX, Castro RA, et al. The effect of milrinone on platelet activation as determined by TEG platelet mapping. *Anesth Analg* 2009;108(5):1425-9.
152. Kikura M, Lee MK, Safon RA, et al. The effects of milrinone on platelets in patients undergoing cardiac surgery. *Anesth Analg* 1995;81(1):44-8.
153. Tanaka H, Tajimi K, Miyajima Y, et al. Effects of milrinone on platelet aggregation in swine with pulmonary hypertension. *J Crit Care* 2000;15(3):113-8.
154. Schermuly RT, Dony E, Ghofrani HA, et al. Reversal of experimental pulmonary hypertension by PDGF inhibition. *J Clin Invest* 2005;115(10):2811-21.
155. Karaman MW, Herrgard S, Treiber DK, et al. A quantitative analysis of kinase inhibitor selectivity. *Nat Biotechnol* 2008;26(1):127-32.
156. Ghofrani HA, Seeger W, Grimminger F. Imatinib for the treatment of pulmonary arterial hypertension. *N Engl J Med* 2005;353(13):1412-3.
157. Patterson KC, Weissmann A, Ahmadi T, Farber HW. Imatinib mesylate in the treatment of refractory idiopathic pulmonary arterial hypertension. *Ann Intern Med* 2006;145(2):152-3.
158. Souza R, Sitbon O, Parent F, et al. Long term imatinib treatment in pulmonary arterial hypertension. *Thorax* 2006;61(8):736.
159. Ghofrani HA, Barst RJ, Benza RL, et al. Future perspectives for the treatment of pulmonary arterial hypertension. *J Am Coll Cardiol* 2009;54(1 Suppl):S108-17.
160. Klein M, Schermuly RT, Ellinghaus P, et al. Combined tyrosine and serine/threonine kinase inhibition by sorafenib prevents progression of experimental pulmonary hypertension and myocardial remodeling. *Circulation* 2008;118(20):2081-9.