This article is intended to deliver a clinically relevant overview about the role of coagulation factors and platelets in the pathogenesis of pulmonary hypertension. After summarizing the available data with warfarin, some information is provided about the novel oral anticoagulants that were recently approved for atrial fibrillation and may soon be approved for venous thrombosis. The author is hopeful that this information will stimulate investigator interest in this topic and drive us toward meaningful studies about this important aspect of PAH therapy.

Pulmonary arterial hypertension (PAH) is characterized by the marked elevation of pulmonary vascular resistance and a reduction in compliance. Vasomotor tone is increased throughout the pulmonary vascular bed, and small and medium arteries are occluded by vascular and inflammatory cells. The progressive loss of the pulmonary circulation leads to exertional dyspnea, low cardiac output, and right ventricular heart failure.

HEALTHY HEMOSTASIS

Vascular cell membrane proteins interact with soluble coagulation proteases to protect the organism from thrombosis and hemorrhage. Tissue factor (TF) is active in the adventitia of healthy blood vessels but much less present in the smooth muscle and endothelium. When TF interacts with circulating factor VII because of vessel injury (eg, in trauma), coagulation is triggered (Figure) as a cascade of serine proteases amplify the signal that first generates activated factor X and ultimately thrombin. Thrombin cleaves fibrinogen to generate an initial fibrin clot, which must then mature into a cross-linked form. Although a full review of coagulation is well beyond the scope of this article, a few key modulators are worth mentioning, as they have been found to be altered in PAH. Thrombomodulin binds thrombin to dampen coagulation, and plasmin cleaves fibrin to limit the propagation of a cross-linked fibrin clot. The endothelium tightly regulates coagulation by synthesizing thrombomodulin and tissue plasminogen activator (t-PA), the latter of which generates local plasmin at the endothelial surface. Thus, the healthy endothelium expresses anticoagulant proteins (thrombomodulin and t-PA) to prevent the formation of a fibrin clot on the surface.

PATHOLOGIC EVIDENCE FOR THROMBOSIS IN PAH

In situ thrombosis occurs in human PAH, and warfarin-based anticoagulation has been associated with improved outcomes in uncontrolled studies. Reduced plasma fibrinolysis (a tendency for fibrin clots to resist degradation, one potential marker of increased tendency for blood coagulation) was first reported in 1973, and a potential causative role for thrombosis in the disease was convincingly proposed in the early 1980s. In that retrospective study of 56 patients who met clinical criteria for primary (now idiopathic) pulmonary hypertension and ultimately underwent autopsy, remarkably the main pathology observed in 50% was thromboembolic type change. Anticoagulation was associated with a more favorable outcome. A second more rigorous autopsy series examined specimens from 58 patients in the initial National Heart, Lung and Blood Institute (NHLBI) Primary Pulmonary Hypertension (PPH) Registry. Nineteen of these 58 patients had thrombotic lesions. Re-canalized thrombi were observed in 9 of 25 patients with plexiform lesions despite the fact that the diagnostic algorithm for the PPH Registry excluded patients with a clinical diagnosis of chronic thromboembolic disease. Autopsy materials from 78 PPH patients in a different study also found frequent thrombotic lesions, and there is now little doubt that in situ thrombosis is a consistent feature of patients with PAH. The topic was recently reviewed.

EVIDENCE FOR ALTERED COAGULATION IN PAH

Increased Thrombin Generation

After the first report of reduced fibrinolysis, many different groups have studied various aspects of the coagulation cascade in plasma from PAH patients. In 1990 (before the introduction of approved therapy or widespread anticoagulation), Rich and colleagues demonstrated elevated levels of fibrinopeptide-A (FPA) in a cohort of 31 patients. FPA generation occurs when thrombin cleaves fibrinogen, and thus these patients apparently had elevated plasma thrombin activity. Intravenous heparin (5000 units) dramatically reduced FPA levels 15 minutes after bolus injection, and Rich proposed that such a technique might be useful in determining whether to treat individual patients with anticoagulation. A smaller study did not confirm the FPA data, but a recent investigation using a more direct measurement of thrombin in 16 treatment-naive PAH patients demonstrated evidence for increased thrombin activity.

Tissue Factor As a Source of Increased Thrombin Activity

TF is a transmembrane glycoprotein that initiates the coagulation cascade and may also participate in angiogenesis and cancer metastasis. TF binds to factor VII to catalyze the activation of factor X leading to the generation of thrombin and the formation of a fibrin clot (Figure). TF
expression is sensitive to changes in blood flow, hypoxia, growth factors (PDGF), and the chemokine MCP-1, all of which are thought to be involved in the pathogenesis of PAH. TF is not present in the endothelial or smooth muscle layer of...
normal vessels, systemic or pulmonary. Studies using inhibitors of TF activity have provided evidence that TF causes injury-related thrombosis in the systemic circulation (via fibrin clot formation).20,21 In collaboration with Carlyne Cool in Denver, we have shown that TF is upregulated in the diseased vessels of PAH patients (surgical or autopsy specimens),22 and another group identified thrombus-promoting, TF-expressing endothelial microparticles in the circulation of PAH patients.23 TF expression on the diseased PAH pulmonary endothelium may be a key contributor to in situ thrombosis and the source of the excess thrombin activity found in the circulation of these patients.

**Reduced Endothelial Thrombomodulin**

Membrane-bound and soluble thrombomodulin (CD141) serves as a thrombin receptor and natural inhibitor of thrombin activity. When thrombomodulin binds thrombin, thrombomodulin also catalyzes the formation of activated protein C to dampen further thrombin production. Circulating thrombomodulin has been consistently low in small groups of PAH patients.24-26 and one of the groups found that increased thrombomodulin levels were associated with continuous prostacyclin therapy in a cohort of 18 patients.25 Low endothelial expression of thrombomodulin would leave thrombin activity relatively unchecked (because thrombomodulin binds to inactivate thrombin).

**Impaired Fibrinolysis**

Measurements of the protein plasminogen activator inhibitor (PAI) have consistently demonstrated elevated PAI levels and/or prolonged euglobin lysis times.24,27-30 Activated plasmin (formed from plasminogen) degrades fibrin clots, and thus elevated levels of PAI (an inhibitor of plasmin generation) would allow fibrin clots to propagate and become more established in the pulmonary circulation. Euglobin lysis time is one way to measure the function of the fibrinolytic system in vivo, and impaired fibrinolysis was the first and is still one of the most consistent findings from different studies in the available literature on coagulation in PAH patients.

In summary, studies of the plasma from PAH patients have found that the stimulus to form clots (TF activity and subsequent thrombin generation) is overactive. A key regulator of normal thrombin activity (thrombomodulin) is reduced, allowing thrombin to “dominate” the more natural equilibrium and push the coagulation cascade at the endothelial surface toward thrombosis. Finally, once thrombin has driven the system to form a fibrin clot, the clot is more likely to endure and propagate because PAH patients have impaired fibrinolytic systems. This impaired system to degrade fibrin clots has been consistently related to elevated levels of a key inhibitor of fibrinolysis, PAI (see Figure).

**EXCESS PLATELET AGGREGATION AND ACTIVATION IN PAH**

Abnormal platelet turnover and the presence of a platelet-derived vasoconstrictor were recognized in the 1970s.31,32 The platelet-derived vasoconstrictor was quickly recognized as thromboxane A2,33 and subsequent measurements of thromboxane metabolites in human PAH patients demonstrated markedly elevated thromboxane with a corresponding reduction in prostacyclin metabolites34; this discovery was made at about the time (1992) that the pivotal trial for epoprostenol was being planned. Two different groups documented abnormal platelet aggregation (both in vivo and after in vitro stimulation).35,36 and subsequently Barst and colleagues demonstrated that abnormal platelet aggregation was less apparent after 1 year of continuous epoprostenol.37

It is now reasonably well established that platelets from PAH patients have excess tendency to aggregate and further that they demonstrate markers of platelet activation.

More recent mechanistic studies shed additional light on the potential role that platelets might play in disease. Platelets are the major source of soluble CD40 ligand in the plasma. In a detailed set of studies on a small number of treatment-naive PAH patients, circulating CD40 ligand was higher than in a group of controls. In freshly isolated platelets from the patients, basal and thrombin-stimulated release of CD40 ligand was greater than controls suggesting that platelets from PAH patients are “primed” to activate in response to thrombin.38 Thus, in addition to evidence for excess thrombin activity in PAH plasma, the platelet itself appears to demonstrate an exaggerated response to thrombin. CD40 ligand stimulates vascular inflammation, and excess CD40 ligand would likely cause further inflammatory-mediated damage to an already injured PAH endothelium. This may be one key way in which activated platelets contribute to PAH pathogenesis beyond the release of well-established mediators like thromboxane A233 and serotonin.39

Interestingly, blood from the pulmonary vasculature of PAH patients contains higher levels of the megakaryocyte-stimulating hormone thrombopoietin, and in one small study, the pulmonary vasculature itself seemed to be a site of production for thrombopoietin.40 There is evidence that the lung is a site of platelet production by megakaryocytes,41 raising the intriguing possibility that the PAH lung facilitates the platelet production that further contributes to disease in a vicious cycle of excess platelet production and activation.

**ANTICOAGULATION IN PAH**

The issue of anticoagulation and survival was addressed in a prospective study of calcium channel blockers for the treatment of PAH. Although the study was not specifically designed to evaluate for the effect of warfarin, a post-hoc analysis revealed that warfarin anticoagulation was associated with a survival advantage ($P=0.025$).2 At the time of this study’s publication, there were already data to support the idea that thrombosis was central to the disease pathogenesis and that anticoagulation was associated with improved survival.1 Because there was no approved therapy at the time and there was already general support for the idea that anticoagulation might be beneficial, this post-hoc analysis of a prospective but nonrandomized study made warfarin anticoagulation commonplace in the management of PAH. A recent retrospective cohort analysis performed at Columbia Presbyterian (NY) examined a consecu-
tive group of patients treated in the prostacyclin era (1994-2002), and confirmed that warfarin was again associated with a reduced mortality. A comprehensive qualitative analysis of this literature suggested that there might indeed be a mortality benefit associated with anticoagulation in idiopathic PAH patients, but that review also put the real risks of major hemorrhage into appropriate context and called for a randomized controlled trial to test the current practice of warfarin anticoagulation. Consensus guidelines acknowledge the weak data but make a strong recommendation for warfarin, especially in the idiopathic patients. The intensity of recommended warfarin anticoagulation is generally less intense in North America (international normalized ratio [INR] 1.7-2.5) than in Europe, where more standard INR of 2-3 is often targeted.

ANTIPATELET THERAPY IN PAH

One small but well-designed study of aspirin (used as a platelet inhibitor) did not demonstrate an improvement in exercise tolerance as measured by 6-minute walk test (6MWT) over 6 months. This multicenter, placebo-controlled trial randomized 65 patients (1:1 aspirin 81 mg or matching placebo) out of a planned 120; the trial was terminated early by the sponsor for futility. Aspirin was effective at reducing thromboxane-A2 levels in comparison with placebo patients, but it was not as effective at reducing markers of platelet activation like P-selectin and β-thromboglobulin. Interestingly, there must be some substantial individual variability in the platelet response to aspirin, because a previous trial with 19 subjects by the same investigators had suggested that 81 mg aspirin was effective at suppressing platelet activation (more so than clopidogrel). Even though this most recent trial was small, the 6MWT in aspirin-treated subjects was identical to placebo; it seems reasonable to draw the firm conclusion that thromboxane did not contribute substantially to exercise intolerance. On the other hand, the ASA treatment did not appear to block platelet activation, and platelet activation itself (as opposed to thromboxane) may be the more important contributor to vascular inflammation and injury. It is also conceivable that platelet activation contributes more to long-term disease progression than short-term exercise intolerance. It thus remains possible that a longer trial, a more efficacious inhibitor of platelet activation (eg, prasugrel), or a more sensitive measure of vascular injury and disease progression might reveal a role for platelet inhibitors in the treatment of PAH.

Clinicians in the year 2012 are thus faced with a significant dilemma. An increasingly older and more heterogeneous population is presenting for PAH care. Warfarin already has a narrow therapeutic index, and although otherwise healthy idiopathic female patients at age 28 may not have exaggerated risk for serious bleeding, many patients obviously have more significant risk. In particular, older idiopathic patients and scleroderma patients with mucosal telangiectasias are certainly at significant risk for the morbidity and mortality associated with warfarin anticoagulation. Truly enormous trials were required to demonstrate that warfarin provided an overall benefit to patients with atrial fibrillation; this was not because the effect size was necessarily small, but rather because the benefit had to be measured against the significant morbidity and mortality. When patients ask about the benefits of warfarin, one must advise cautiously and provide the risks as well as the potential benefits. The truth is that we have no convincing evidence that the risks associated with warfarin are outweighed by the benefits. The guidelines are consistent in recommending warfarin based on the limited data outlined above, but the risks have never been quantified.

MORE THAN CLOT

Despite the somewhat dim view of warfarin outlined above, it is critical to remember that we have excellent scientific rationale to evaluate targeted anticoagulants in PAH. Fibrin clots may in fact be a “bystander” marking the thrombin activity, much like yellow nails mark the consequences of long-term tobacco use but have little to do with lung cancer. Warfarin dampens the formation of fibrin clots by inhibiting the synthesis of prothrombin and factors VII, IX, and X. Warfarin thus causes a global but modest suppression in these signaling cascades that ultimately results in a significant reduction in the formation of fibrin clots in relatively static areas of the circulation. However, thrombin generation may still be substantial even with warfarin dosing that is effective at reducing fibrin clots. One relatively large study (n = 134) demonstrated that patients with similar levels of INR had very different levels of plasma thrombin generation. Especially because we often target relatively lower INR in our PAH patients, it is entirely possible that many individuals still have very active thrombin generation.

Factor Xa and thrombin are critical molecular signals in the vasculature (see Figure). These serine proteases (Xa and thrombin) act on a unique class of receptors, the protease activated receptors (PAR). PAR are G-protein coupled, cell-surface receptors that, once activated, signal much the same as other more familiar G-protein coupled receptors (eg, the beta-adrenergic or prostacyclin receptors). In humans, PAR1 is the classic thrombin receptor on platelets, endothelial, and smooth muscle cells. Factor Xa likely acts at PAR1 and PAR2. Activation of PAR1 and PAR2 recruits inflammatory cells to the vascular wall, increases endothelial cell permeability, and promotes smooth muscle cell migration and hyperplasia. Therefore, activation of these receptors promotes pathologic processes, which are already known contributors to the vascular biology of PAH.

TF AND SYSTEMIC ARTERIAL INJURY

A variety of different kinds of TF inhibitors have been shown to reduce long-term arterial injury and remodeling in diseases of the systemic circulation, such as coronary artery ligation and carotid artery injury. Importantly, arterial injury in many of these studies (particularly those involving rodents, reviewed in) is associated with only small amounts of thrombus, strongly suggesting that TF with down-
stream Xa and thrombin formation mediates changes in the arterial wall independent of fibrin clot formation (reviewed in 52,53). Given the severity of arterial remodeling and the presence of thrombosis in the pulmonary vasculature in patients with PAH, some experts hypothesize that TF, Xa, and thrombin play an important role in the progression of PAH, just as they do in the progression of vascular remodeling on the systemic arterial side.

Warfarin targeted to an INR of 2-3 definitely reduces the formation of fibrin clots, especially in the venous system or areas of stasis. However, with lower INR targets, the degree to which warfarin attenuates Xa/thrombin generation and PAR activation in the pulmonary arteries of our PAH patients has never been studied but is almost certainly variable. Thus, it would be quite appropriate to test the hypothesis that PAH patients would benefit from targeted anticoagulants, which more directly inhibit vascular PAR activation (and fibrin clot formation).

**NOVEL ANTICOAGULANTS**

Dabigatran (Pradaxa), an oral direct thrombin inhibitor (see Figure), was recently approved for the prevention of stroke in patients with atrial fibrillation. It is also European Medicines Agency (EMEA) approved for the prevention of venous thromboembolism following orthopedic procedures. Dabigatran at 150 mg BID was more effective than warfarin in reducing stroke with similar rate of major bleeding (although more significant gastrointestinal bleeding). One trial was favorable for treating venous thromboembolism and another is in progress.54

Rivaroxaban (Xarelto) is an oral Factor Xa inhibitor that is approved for stroke prevention and as prophylaxis against venous thrombosis in orthopedic patients.54 This once-daily drug has more recently been demonstrated as a safe and effective treatment for deep venous thrombosis55 and pulmonary embolism56 and as adjunctive therapy for patients with acute coronary syndromes.57 Fatal and intracranial hemorrhage was less frequent with rivaroxaban than with warfarin in the double-blind, double-dummy atrial fibrillation trials.

These novel agents don’t require regular monitoring, and they are less sensitive to food-drug or drug-drug interactions than warfarin. Both drugs have been studied in tens of thousands of patients in multiple different populations,54 and so the pharmacokinetic characteristics (especially in patients with mild renal or liver insufficiency) are well described. As a caution, there is no specific antidote for the anticoagulation effects of either compound, although prothrombin concentrations would likely prove effective.

Thus, there are now two approved and well-studied drugs with which to test the hypothesis that targeted anticoagulation would be beneficial for PAH patients. Several other compounds are in late-stage development and will likely soon be approved. A placebo-controlled, randomized trial with one of these agents would answer critical questions about the safety of anticoagulation in these fragile patients and would firmly establish the risk-benefit relationship so that clinicians could offer informed advice to individual patients before prescribing anticoagulation. Indeed, pulmonary hypertension patients face enough therapeutic complexity in their day-to-day lives; if practitioners are going to help them make meaningful decisions about a therapy with real risk, they must have firm data about the long-term advantages and the likely problems. A large trial that included associated PAH patients (eg, scleroderma) would also help the PH community to understand whether certain populations were more likely to enjoy benefit or experience risk.

**CONCLUSION**

In summary, the literature is replete with data to suggest that in situ thrombosis occurs in PAH and that consistent plasma coagulation abnormalities can be measured in many PAH patients. Excess platelet activation and turnover have been repeatedly demonstrated, and some of these plasma coagulation and platelet abnormalities appear sensitive to continuous prostacyclin therapy. Warfarin has been associated with a survival benefit in both prospective and retrospective analyses of idiopathic PAH, but there has never been a randomized study of warfarin. Moreover, there is no meaningful collection of the short- or long-term risks associated with warfarin, especially for patients already on therapies that inhibit platelet function and may therefore predispose patients to bleeding. The introduction of direct thrombin and Xa inhibitors offers an unprecedented opportunity to study the risks and benefits of targeted anticoagulation. By highlighting proposed mechanisms of thrombosis and vascular injury in PAH patients and the potential therapeutic targets, one can see the tremendous opportunity for future investigations in this arena. Hopefully, we can bridge the current gap between the lab and routine clinical application.

**References**

CME

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