Special Review

Pulmonary Arterial Hypertension

Future Directions

Report of a National Heart, Lung and Blood Institute/Office of Rare Diseases Workshop*

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Pulmonary arterial hypertension (PAH) is characterized by vascular obstruction and the variable presence of vasoconstriction, leading to increased pulmonary vascular resistance and right-sided heart failure. PAH can present in an idiopathic form, usually called primary pulmonary hypertension (PPH), and PAH is also associated with the scleroderma spectrum of diseases, HIV infection, portal hypertension with or without cirrhosis, and anorectic drug ingestion. Idiopathic PAH occurs in women more often than men (>2:1), has a mean age at diagnosis of 36 years, and is usually fatal within 3 years if untreated. Modern treatment has markedly improved physical function and has extended survival, and the 5-year mortality rate is ~50%. We still do not understand what initiates the disease or what allows it to progress. New studies of the pathogenetic basis of PAH will lead to targeted therapies for PAH. The National Heart, Lung and Blood Institute (NHLBI) and the Office of Rare Diseases (ORD), National Institutes of Health, convened a workshop to bring together investigators with various interests in vascular biology and pulmonary hypertension to identify new research directions. Discussion included genetics of PAH, receptor function, mediators, ion channels, extracellular matrix, signaling, and potential clinical approaches.

Background and Questions

Molecular genetic studies have demonstrated mutations in a receptor in the transforming growth factor (TGF-β) superfamily, called bone morphogenetic protein receptor 2 (BMPR2), in most cases of familial pulmonary hypertension.1,2 Less common mutations associated with PAH occur in Alk1, a TGF receptor that also causes hereditary hemorrhagic telangiectasia.3 Because only ~10% to 20% of persons with a BMPR2 mutation develop PAH, it is likely that other genes, genetic polymorphisms, and environmental factors are necessary to initiate the pathological sequence that leads to disease.4 Most cases of PAH are not associated with known inherited genetic mutations.5 Thus, external stimuli coupled with as-yet-undefined genetic susceptibility to disease are...
likely responsible for most cases of PAH. The abnormal transduction of signals related to BMPR2 and Alk/endoglin is unknown and needs aggressive investigation. This will involve understanding of extracellular stimuli, receptor and membrane channel responses, and activation of a variety of intracellular molecules such as SMAD proteins, mitogen-activated protein (MAP) kinases, and nuclear transcription factors.6

Several basic questions are unanswered. First, is there a common final pathway that is activated in response to a variety of disease-producing stimuli, or are there multiple independent pathways? Second, what cell or cells initiate the process in the vascular bed? Third, how does a mutated BMPR2 or Alk1 fail to suppress cellular abnormalities? Fourth, why are women at higher risk for disease? Finally, are affected vessels subject to an autonomous process, or does the disease require ongoing stimuli and therefore have the potential to be reversed? Multimodality therapy needs to be considered for clinical study, especially with endothelin A receptor antagonists and phosphodiesterase 5 inhibitors. Drugs known to protect the systemic vascular bed, such as the HMG Co-A reductase inhibitors, are candidates for treatment trials. Other drugs, such as elastase inhibitors, that have efficacy in experimental disease in animals need to be explored.7

Pathogenesis

The Figure depicts the biological milieu from which abnormal pulmonary vascular responses might lead to PPH. The figure is necessarily simplified and serves only as a guide for discussion of pathogenesis. Discussion of potential therapies will interdigitate with mechanisms of disease.

BMPR2 and Alk/Endoglin Mutations

Multiple loss-of-function mutations have been described in BMPR2 and ALK1.1-2,3,5 Activation of the TGF-β-BMPR2 axis leads to suppression of proliferation and activation of apoptosis; conversely, these loss-of-function mutations exaggerate the susceptibility of vascular cells to proliferate.8 Somatic mutations in endothelial cells microdissected from plexogenic lesions are found within the human MutS Homolog 2 gene that lead to reduced protein expression of TGF-β and thus suppression of apoptosis.9 Furthermore, pulmonary artery smooth muscle cells (PASMCs) from BMPR-knockout mice have abnormally enhanced proliferation rates in response to growth factors in vitro.10 The extensive diversity and tissue specificity of the SMAD system and the heteromultimeric formation of different TGF/BMP receptor subtypes may explain the localization of the disease to the small pulmonary arteries.
**K⁺ Channels, Vascular Tone, and Proliferation**

Vasoconstriction is a feature in some cases of pulmonary hypertension, and mechanisms relevant to PPH might exist in the pulmonary response to hypoxia. Hypoxia inhibits ≥1 voltage-gated potassium channels (Kᵥ) in the PASMCs, opening voltage-gated calcium channels, raising cytosolic Ca²⁺, and initiating constriction.¹¹

Kᵥ1.5 or Kᵥ2.1 channels are downregulated in the PASMCs in humans with PAH¹² and in rats with chronic hypoxia-induced pulmonary hypertension.¹³ Furthermore, DNA microarray studies have shown downregulation of Kᵥ channel genes in lungs of patients with PAH. In contrast, the genes for inward rectifier potassium channels (Kir) are upregulated in PAH.¹⁴ Whether these Kᵥ channel abnormalities are genetically determined or acquired is unknown, but they are not present in secondary pulmonary hypertension. It is unknown whether these PASMC Kᵥ channel abnormalities are related to the TGFβ2 abnormalities that have been described mostly in pulmonary artery endothelial cells.⁹ However, it is clear that the anorexigens dexfenfluramine and aminorex are K⁺ channel blockers.¹⁵

There is also a link between K⁺ channels and vascular remodeling through apoptosis, which may be relevant to PAH. Yuan et al have observed that agents that activate KᵥCa and Kᵥ channels, such as nitric oxide (NO), increase K⁺ efflux, which leads to cytosolic K⁺ loss, volume decrease, and apoptosis.¹⁶ It has been hypothesized that PAH could also be viewed as a "K⁺ channelopathy" in which loss of channels (acquired or genetic) leads to vasoconstriction, cell proliferation, and loss of basal apoptosis.¹⁷

Modulation of K⁺ channel function may have therapeutic potential. Augmenting the K⁺ channels should cause pulmonary vasodilatation and regression of pulmonary artery remodeling. Several oral treatments such as dichloroacetate and sildenafil may be able to enhance the function of these K⁺ channels.¹⁸ Sildenafil and other PDES inhibitors cause pulmonary vasodilatation in large part through the BKC₉₅ mechanism. Oral dichloroacetate, a metabolic modulator, increases expression/function of Kᵥ2.1 channels and decreases remodeling and pulmonary vascular resistance in rats with hypoxic pulmonary hypertension, partially via a tyrosine kinase-dependent mechanism.¹⁸ Dichloroacetate appears safe in humans (according to prior heart failure studies) and might be useful in treatment of PAH.

**Statins and Mechanisms of PPH**

The HMG-CoA reductase inhibitors statins confer potent antiproliferative and antiinflammatory cardiovascular benefit, in addition to cholesterol-lowering effects.¹⁹,²⁰ Statins suppress endothelial and vascular smooth muscle cell neointimal responses to vascular injury in animal models.²¹,²² Among the mechanisms of statin actions is inhibition of the isoprenylation of rho and ras family GTPases that couple membrane growth factor receptors to the intracellular MAP/ERK kinase signaling pathways that influence proliferation²³ (Figure). Additionally, statins augment endothelium-dependent NO production and vasodilation through stabilization of endothelial NO synthase mRNA.²⁴ Furthermore, statin enhancement of Akt kinase increases circulating endothelial progenitor cells that may contribute to vascular repair.²⁵

In a monocrotaline rat model of PAH, simvastatin attenuated and reversed both pulmonary hypertension and neointimal formation and improved survival from 0% to 100%. Simvastatin reversed vascular occlusion through reduced intimal proliferation and increased apoptosis of pathological smooth muscle cells in pulmonary arteries.²⁶ Similar results were recently noted in a rat model of hypoxic pulmonary hypertension.²⁷ These data suggest that statins should be evaluated for treatment of patients with PPH and possibly for prevention in susceptible individuals.

**Elastase Inhibitors and Regression of PAH**

In rats subjected to hypoxia or monocrotaline, serine elastase increases in the pulmonary arteries before vascular remodeling, related to phosphorylation of MAP kinase and induction of AML1-transactivating activity.²⁸,²⁹ Inhibition of elastase attenuates pulmonary hypertension and structural changes. Elastase activates matrix metalloproteinases, which amplify proteolytic response in the vessel wall and can release growth factors from the matrix in a biologically active form.³⁰ The mitogenic potential of these growth factors is enhanced by elastase-MMP-mediated induction of the glycoprotein tenascin-C via β-3 integrin signaling.³¹ Tenascin amplifies the response to growth factors such as epidermal growth factor by inducing phosphorylation of growth factor receptors. In a monocrotaline rat model, elastase inhibition resulted in 86% survival compared with 100% mortality, plus regression of structural changes and pulmonary hypertension.³² Thus, elastase inhibitors may have promise in the treatment of clinical disease.

**Nitric Oxide**

NO is a potent pulmonary vasodilator, has antiplatelet activity, interacts with reactive oxygen species, and protects K-channel function.³³ L-Arginine, the sole substrate for NO synthase, can be reduced by pregnancy or stress.³⁴ Exogenous arginine seems to increase NO production. In endothelium, the arginine transporter is tightly colocalized with NO synthase.³⁵ If the arginine transporter is disrupted by low-level endothelial injury, extracellular levels of arginine might become insufficient.

Arginine is an effective NO donor in the treatment of acute sickle cell lung crisis.³⁶ Efforts to improve pulmonary hemodynamics in adults with pulmonary vascular disease with arginine have met with mixed results.³⁷,³⁸ Whether chronic arginine supplementation can improve the lung circulation in patients with PAH is unknown. It is possible that the coadministration of oral arginine or other NO donors with standard therapies would result in additive effects.

**Serotonin (5-Hydroxytryptamine)**

5-Hydroxytryptamine (5-HT) has been implicated in the pathogenesis of PAH. The 2 most likely mechanisms are vasoconstriction and a mitogenic effect.³⁹,⁴⁰ Compared with control subjects, patients with PPH have decreased platelet 5-HT, increased plasma 5-HT concentration, and increased release during platelet aggregation. Plasma 5-HT levels are also elevated in patients with fenfluramine-induced PAH.⁴⁰
Dexfenfluramine releases 5-HT from platelets, inhibits reuptake, and causes inhibition of voltage-sensitive (Kv) channels, membrane depolarization, and calcium entry into PASMCs and megakaryocytes. These drugs are also serotonin transporter substrates and may interfere with intracellular signaling. The 1-allelic variant of 5-HT transporter gene promoter, associated with 5-HT transporter overexpression and increased PASMC growth, is present in homozygous form in 65% of PPH patients and in 27% of control subjects. Thus, a 5-HT transporter polymorphism may confer susceptibility to PPH.

5-HT promotes PASMC hyperplasia through the serotonin transporter via production of reactive oxygen species and MAP kinase activation. PASMCs from PPH patients grow faster than those from control subjects when stimulated by 5-HT because of increased expression of the serotonin transporter. In cultured rat PASMCs, 5-HT potentiates the mitogenic effect of platelet-derived growth factor-BB. 5-HT transporter inhibitors eliminate the difference between PPH patients and control subjects in PASMC growth responses.

Exploration of agents that inhibit 5-HT transporter (such as fluoxetine or paroxetine), reduce platelet aggregation and serotonin release (such as aspirin), or block serotonin receptors involved in vasoconstriction (such as ketanserin) in the management of PAH is warranted.

Endothelin
Endothelin, an endogenous peptide, is a powerful pulmonary vasoconstrictor with mitogenic and fibrogenic effects. It is elevated in the blood in PAH. The vasoconstrictor effects are mediated by Ca\(^{2+}\) channel activation and influx, and the growth and repair signals are transduced by activation of MAP kinases, including ERK and Jun, via G-proteins. Endothelin receptor antagonists are highly effective in some patients with PAH and have become important therapies. The intracellular signaling pathways of endothelin interact with several other of the mediators of interest in PAH (Figure).

Platelets and Antiplatelet Therapy
Platelets present 5-HT, thromboxanes, and platelet-derived growth factor to the vascular wall, but very little is known about the effects of antiplatelet therapy in PAH. Some of the long-term benefits of prostacyclin analogs in the treatment of PAH might be from antiplatelet activity and the vasodilator and possible inotropic effects. The clinical potential of primary antiplatelet agents has not been formally studied in PAH, in contrast to their proven efficacy in systemic vascular disease. Aspirin therapy is particularly attractive in PAH, in part because of its reduction in platelet thromboxane production. Thromboxanes are elevated in patients with PAH, and prostacyclin and prostacyclin synthase are decreased in PAH.

A favorable effect of anticoagulant therapy with warfarin in PAH is generally accepted although based on only 3 relatively small studies. It is unknown whether aspirin or other antiplatelet therapy would demonstrate similar benefit with less risk and reduced need for monitoring.

Genetic Approaches
PPH is a complex genetic disease, meaning that gene–gene and environment–gene interactions may confer susceptibility to disease. Approaches to discovering modifying genes will involve studies of PAH patients for underlying polymorphisms such as in the serotonin transporter. A new approach is the development of a hypertensive phenotype in transgenic mice with hypoxia, drugs, other stimuli, or other underlying genetic backgrounds. The search for modifying genes will involve known candidate genes such as NOS plus genome-wide surveys. Studies of the effects of polymorphisms are underway for many of the mediators shown in the Figure.

Genome-wide searches with SNP analysis, reverse-transcriptase polymerase chain reaction and microsatellite markers will require large cohorts and extensive resources to complete. Information from cDNA arrays and clusters and proteomic translation will be useful to determine the patho-genetic spectrum of disease in microdissected lesions and in stimulated cell and tissue experiments. At this time, little evidence about modifying genes has been published, so this field is young and full of promise.

Discussion About Therapy
Therapeutic advances over the past 2 decades have improved the natural history of PPH and of PAH arising from other causes, including the scleroderma spectrum of disease, Eisenmenger’s syndrome, HIV, anorectic drug use, and portal hypertension. Current medical therapy includes supportive treatment, eg, digitalis, diuretics, and supplemental oxygen; anticoagulation (warfarin); calcium channel blockade (in the minority of patients with sustained vasodilation); chronic intravenous epoprostenol; newer PGI2 formulations (intravenous or inhaled iloprost, subcutaneous, aerosol, and intravenous treprostinil, or oral beraprost); and endothelin receptor antagonists. Despite these advances, PAH remains a devastating disease, and most approved therapies are very expensive and offer minor benefits to exercise capacity. Thus, there is a strong rationale to consider a number of novel therapies related to pathogenic mechanisms. These include, but are not limited to, phosphodiesterase inhibitors, statins, L-arginine, antiplatelet agents, serotonin inhibitors, agents to alter ion channel function, gene therapy, VIP, elastase inhibitors, antiproliferative heparins, and possibly tyrosine kinase inhibitors. Multimodal/combination therapies may also further improve PPH treatment but need critical evaluation in prospective, controlled trials. A major priority should be the discovery of new biomarkers that permit noninvasive diagnosis and monitoring of PPH and other forms of PAH.

The workshop was not constituted to propose clinical trials but rather to find early leads for targeted therapy for future clinical applications. An excellent discussion of clinical trials in PPH has been recently published.

Future Research Approaches and Recommendations
The workshop discussions reflected the current momentum and excitement surrounding research on PAH. The field is in a data-gathering phase because of application of new technology to the pulmonary circulation. New information will facilitate collaboration between basic scientists and clinical
investigators and will accelerate translation to clinical care. Recommendations for research directions and opportunities are as follows.

**Genetic Studies**

1. Establish an international blood and tissue bank for PAH that will have wide access for genome, proteomic, biomarker, and histological studies.
2. Support sequencing of the complete BMPR2 gene in patients without known predisposing mutations and the search for other major genes causing heritable PPH.
3. Screen BMPR2 mutation–positive families for genes that modify the penetrance of disease using genome-wide searches and new techniques of statistical genetics.
4. Support functional studies of likely candidate modifier genes (eg, serotonin transporter, NO synthase, and VIP).
5. Transgenic mice and transfected cells are important models for testing biological effects of altered genes and for therapies and need further implementation.

**Receptors, Mediators, Ion Channels, and Signaling Studies**

1. Studies of cellular responses to growth factors and interactions of intracellular signaling molecules, including MAP kinases, reactive oxygen species, G protein–coupled agents, vascular endothelial growth factor, and tyrosine kinases.
2. Studies of apoptosis in PAH endothelia and smooth muscle cells, including the roles and interaction of K channels, NO, statins, serotonin, reactive oxygen species, and BMP2R signaling.
3. Use of gene array and proteomic expression to detect clusters of tissue and blood cell responses in PPH tissues, transgenic animals, and cell systems.
4. Studies of control of matrix formation and function and interactions with vascular cells to understand development, control, and regression of lesions.

**Clinical Studies**

1. Clinical trials using combinations of drugs with differing mechanisms are needed, including combinations of PDE5 inhibitors, prostacyclin analogs, endothelin antagonists, NO or NO synthase, and antiproliferative agents.
2. A study of efficacy of anticoagulant therapy or comparisons of warfarin versus antiplatelet drugs is warranted.
3. Clinical trials and funding for them should include hypothesis-driven measurement of cellular, tissue, or fluid biomarkers to discern mechanisms of disease and drug effect.
4. Phase 1 trials using K channel openers, antiserotonin drugs, or statins need consideration.

**References**


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