ENTELLIGENCE™

Young Investigators Award Program

The ENTELLIGENCE program is supported through an educational grant from Actelion Pharmaceuticals US, Inc. ENTELLIGENCE is run as a nonprofit program.
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Dear Colleagues,

We are delighted to announce that in 2010, the Actelion ENTELLIGENCE™ Young Investigators Award Program awarded five new pulmonary hypertension-related research projects. These awards provide support to individual young investigators at universities and research institutes in the US and Canada to conduct basic science and clinical research through a 12-month mentored grant. Since 2005, members of the independent Steering Committee have selected cutting-edge projects that are scientifically relevant, original, and applicable to the problem of pulmonary hypertension. More than 20 promising researchers in the field of pulmonary hypertension have been funded.

Original basic and clinical investigations target pulmonary vascular disease in the areas of pathophysiology, pharmacology, treatment, genetics, diagnosis, and epidemiology. Results from many of these projects are presented at key scientific meetings such as the American Thoracic Society and published in peer-reviewed journals, including Chest, American Journal of Respiratory and Critical Care Medicine, and American Journal of Physiology.

Continuing its commitment to advancing the understanding of pulmonary hypertension, the Young Investigators Award Program will soon begin another cycle of competition, with relevant dates announced later this year.

On behalf of the ENTELLIGENCE Steering Committee, I would like to express our gratitude to Actelion for their generous gifts to the pulmonary hypertension research community and their ongoing commitment to basic science and clinical research in this arena.

Best regards,

Ronald J. Oudiz, MD
The ENTELLIGENCE Young Investigators Award Program

Supporting young investigators

The ENTELLIGENCE Young Investigators Award Program, established in 2005, provides opportunities for promising young investigators to promote quality medical care and enhance patients’ lives by supporting research (basic science, clinical, or translational) in the area of pulmonary hypertension (PH), specifically related to expanding knowledge of pulmonary vascular pathobiology pathways. ENTELLIGENCE is led by a Steering Committee comprised of leaders in the field of PH who award 12-month mentored grants of up to $100,000 to conduct basic science and clinical research. Grants are based on scientific merit, originality, feasibility, and applicability to the diagnosis and treatment of PH, conditional upon supervision by an appropriate mentor, and conducted at a university or research institute in the US or Canada. The program is consistent with Actelion’s commitment to basic science and clinical research in the area of PH.

ENTELLIGENCE is funded by an independent grant from Actelion Pharmaceuticals US, Inc. All decisions to fund protocols are solely decided by the ENTELLIGENCE Steering Committee and the receipt of a grant in no way requires the recipient, nor implies that the recipient is obligated to, recommend or prescribe any Actelion product.

How to submit

Applicants are invited to submit original basic or clinical investigations specifically targeting pulmonary vascular disease in the following areas: Pathophysiology, Pharmacology, Treatment, Genetics, Diagnosis, and Epidemiology. Applications are submitted electronically as a Letter of Intent. Submitted applications are reviewed by the Steering Committee and selected applicants are invited to submit full proposals. The timelines, submission procedure, and submission forms are available on the ENTELLIGENCE website (www.entelligencemd.org).

Review cycles completed: 5
Awards distributed: 30
Overview of ENTELLIGENCE Awards

Awarded 2010

**Eric Douglas Austin, MD, MSCI**  
Vanderbilt University Medical Center  
Nashville, Tennessee  
Mentor: James E. Loyd, MD  
*Project Title: Sex Hormone Abnormalities in Pulmonary Arterial Hypertension*

**Angela V. Ghatnekar, PhD**  
Medical University of South Carolina  
Charleston, South Carolina  
Mentor: Richard M. Silver, MD  
*Project Title: The Role of GATA-6 in Pulmonary Arterial Hypertension*

**Jason Gien, MD**  
University of Colorado Health Sciences Center  
Aurora, Colorado  
Mentor: Steven H. Abman, MD  
*Project Title: ET-1-Rho-kinase Interactions in the Pathogenesis of Neonatal Pulmonary Hypertension*

**Michael J. Passineau, PhD**  
Allegheny General Hospital  
Pittsburgh, Pennsylvania  
Mentor: Raymond L. Benza, MD  
*Project Title: Gene Therapy to Drive Endogenous Biosynthesis of Prostacyclin*

**Michael York, MD**  
Boston University Medical Center  
Boston, Massachusetts  
Mentor: Harrison Farber, MD  
*Project Title: dsRNA Stimulates Toll-like Receptor-3 and Increases Endothelin-1 Production by Pulmonary Artery Endothelial Cells*
Overview of ENTELLIGENCE Awards

Awarded 2009

Daniel J. Kass, MD
Columbia University
College of Physicians and Surgeons
Mentor: Jahar Bhattacharya, MBBS, DPhil
Project Title: Targeting the MetAP2 Pathway in Pulmonary Arterial Hypertension

Sean E. McLean, MD
UNC School of Medicine
Mentor: Cam Patterson, MD, MBA
Project Title: Smooth Muscle Cell Related Vascular Remodeling in Pulmonary Hypertension in Congenital Diaphragmatic Hernia

Alexander R. Opotowsky, MD, MPH
Children’s Hospital Boston
Boston Adult Congenital Heart Program
Mentor: Michael J. Landzberg, MD
Project Title: The Epidemiology and Determinants of Hospitalization for Pulmonary Hypertension in the United States

Michael Eric Yeager, PhD
University of Colorado School of Medicine
Mentor: D. Dunbar Ivy, MD
Project Title: Circulating Mesenchymal Precursors in Severe PAH and the Role of Endothelin-1 in their Recruitment and Differentiation into Fibrocytes
Overview of ENTELLIGENCE Awards

2009 Award Winners
Overview of ENTELLIGENCE Awards

Awarded 2008

**Gaurav Choudhary, MD**
Brown University
Mentor: James Klinger, MD
*Project Title: Role of Endothelin-induced PKC delta Activation in Right Ventricular Hypertrophy*

**Hyung J. Chun, MD**
Stanford University School of Medicine
Mentor: Thomas Quertemous, MD
*Project Title: Role of the Apelin-APJ Pathway in Endothelin-1 Signaling and Pulmonary Arterial Hypertension*
Presented at 2009 American Heart Association meeting

**Scott D. Halpern, MD, PhD**
University of Pennsylvania School of Medicine
Mentor: Brian Strom, MD
*Project Title: Racial Differences in Responsiveness to Endothelin Receptor Antagonists in Pulmonary Arterial Hypertension*

**Sayyed A. Hamidi, MD**
State University of New York, Stony Brook
Mentor: Margaret Parker, MD
*Project Title: A New Combination Therapy for Pulmonary Arterial Hypertension: Bosentan and VIP*
Accepted for Presentation at 2010 American Thoracic Society Conference, 2010 Aspen Lung Conference, and 2010 Annual Congress of European Respiratory Society (funded by ENTELLIGENCE and the National Institutes of Health)
Overview of ENTELLIGENCE Awards

Awarded 2008

Sanjiv Shah, MD
Northwestern University Medical Center
Mentor: John Varga, MD

Project Title: Generic Risk Factors for Connective Tissue Disease (CTD)-Associated Pulmonary Arterial Hypertension (PAH)
Published in Current Rheumatology Reports, 2009

Venkataramana Sidhaye, MD
Johns Hopkins University
Mentor: Larissa Shimoda, PhD

Project Title: Endothelin-1 Mediated Pulmonary Smooth Muscle Migration is Mediated by AQP1

Ari Lev Zaiman, MD, PhD
Johns Hopkins University
Mentor: Hal Dietz, MD

Project Title: Role of Endothelin Abrogation of TGF Signaling in the Vascular Endothelium Attenuates Hypoxia Induced Pulmonary Hypertension
Overview of ENTELLIGENCE Awards

Awarded 2007

Yabing Chen, PhD
UAB School of Medicine
Mentor: Raymond Benza, MD
Project Title: PAI-1 Regulates Vascular Remodeling in Hypoxia-Induced Pulmonary Hypertension

Christopher Fiack, MD
John A. Burns School of Medicine
Mentor: Harrison Farber, MD
Project Title: Pulmonary Hypertension due to the Left Ventricular Dysfunction

Anna R. Hemnes, MD
Vanderbilt University School of Medicine
Mentor: John Newman, MD
Project Title: The Role of Endothelin-1 in Right Ventricular Response to Pressure Overload
Presented at 2008 American Thoracic Society Conference

Jeffrey C. Horowitz, MD
University of Michigan Health System
Mentor: Victor J. Thannickal, MD
Project Title: Regulation of Myofibroblast Resistance to Apoptosis by Endothelin-1
Published in American Journal of Respiratory Cell and Molecular Biology, 2009

Meredith A. Preuss, PhD
UAB School of Medicine
Mentor: David Curiel, MD
Project Title: Downstream Redox Regulation of Endothelin B Receptor in the Pulmonary Endothelium
Awarded 2007

Olga Rafikova, PhD, MD
University of Pittsburgh
Mentor: Stevan Tofovic, PhD
Project Title: Protein Nitration and Inhibition of Cardiac and Vascular Remodeling in Pulmonary Hypertension Endothelin Receptor Antagonists

Megha H. Talati, PhD
Vanderbilt University Medical Center
Mentor: Barbara Meyrick, PhD
Project Title: Effect of BMPR2 Mutation in FPAH on ET-1 and ET-1 Receptors and Smad/MAPK Activation by ET-1 Receptors in Lung ECs and PASMCs in the Mouse Model of PAH
Presented at 2009 American Thoracic Society meeting [travel funded by Cardiovascular Medicine Research and Education Fund]

Yerem Yeghiazarians, MD
University of California, San Francisco
Mentor: Teresa DeMarco, MD
Project Title: Effect of Endothelin Receptor Blockade on Circulating Endothelial Microparticle Levels in Patients with Pulmonary Hypertension
Overview of ENTELLIGENCE Awards

Awarded 2006

Joel Glasgow, PhD
UAB School of Medicine
Mentor: David Curiel, MD

Project Title: Gene Delivery for Pulmonary Hypertension

Zhigang Hong, PhD, MD
University of Chicago
Mentor: Kenneth Weir, MD

Project Title: Endothelin-Induced Increase in Pulmonary Vascular Smooth Muscle Calcium; The Role of Calcium Channels


Peter Oishi, MD
UCSF School of Medicine
Mentor: Jeffrey Fineman, MD

Project Title: Endothelin-1 Reactive Oxygen Species Interactions in Pulmonary Hypertension


Rajni Rao, MD
UCSF School of Medicine
Mentor: Yerem Yeghiazarians, MD

Project Title: Quantitative and Qualitataive Properties of Endothelial Progenitor Cells in Patients with Pulmonary Hypertension

Presented at 2007 International Society of Heart and Lung Transplantation meeting
Overview of ENTELLIGENCE Awards

Awarded 2006

Giuseppe Valacchi, PhD
University of Siena
Mentors: Carol Cross, MD, and Gian Paolo Pessina, Professor

Project Title: Does Tocopherol Homeostasis Play a Role in Endothelin Mediated Endothelial Dysfunction?

Roham Zamanian, MD, FCCP
Stanford University School of Medicine
Mentor: Ramona Doyle, MD

Project Title: The Effect of Endothelin A and B Antagonism on Insulin Resistance and Outcomes in Patients with Pulmonary Arterial Hypertension
Sex Hormone Abnormalities in Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is a progressive, fatal disease characterized by increased pulmonary vascular resistance and arterial pressure resulting in right heart failure and rapid death. Most types of PAH, including heritable (HPAH) and idiopathic (IPAH), predominantly affect women for unknown reasons, and our preliminary laboratory data support the central hypothesis that sex hormone variation modifies disease expression in PAH, with metabolites that possess greater estrogenic effects in abundance compared to those with less estrogenic effects. To test our hypothesis, we will determine whether mediators of estrogen and androgen activity are associated with HPAH and IPAH in females and males. We hypothesize that higher estrogen activity (e.g., as represented by a lower ratio of 2-hydroxyestrogens: 16α-hydroxyestrogens) will be associated with increased risk of disease and younger age at diagnosis. We expect this study to confirm and extend the PI’s preliminary studies demonstrating altered sex hormone levels in PAH patients, with implications for disease prevention and therapy.
Our recent data revealed that GATA-6, a zinc-finger transcription factor, is strikingly down-regulated at the mRNA and protein level in endothelial cells (ECs) lining all vessel types, both occluded and non-occluded, in idiopathic pulmonary arterial hypertension (PAH) patients, as well as systemic sclerosis-associated PAH patients. In addition, we have found that GATA-6 transcript levels are reduced in the lungs of the monocrotaline rat model of PAH at an early time point in disease onset. Furthermore, in vitro data suggest that loss of GATA-6 in ECs leads to a reduction in EC markers and an increase in markers of vascular remodeling. Based on our preliminary findings, we hypothesize that loss of GATA-6 after vessel injury may be an important component of EC activation/dysfunction. Moreover, reduction of GATA-6 may be an important early feature of PAH. To study the potential role of GATA-6 in PAH, we propose to investigate the functional consequences of reduced GATA-6 in ECs, both in cultured human pulmonary artery endothelial cells (HPAECs) and in mouse lungs in vivo. In specific aim 1, we will investigate changes in gene expression patterns in HPAECs with reduced GATA-6 using Human Endothelial Cell Biology PCR arrays. Putative direct transcriptional targets will be validated using quantitative RT-PCR, western blotting, and chromatin immunoprecipitation (ChIP) analysis. In specific aim 2, we propose to investigate the functional consequences of decreased GATA-6 levels in pulmonary endothelial cells in vivo using
Angela V. Ghatnekar, PhD

transgenic mice (Cre/loxP system) with conditional GATA-6 knockdown in endothelial cells. GATA-6 conditional knockout mice will be characterized by performing hemodynamic analyses and lung tissue analyses with respect to vascular abnormalities. Lung endothelial cells will also be isolated to check for differential expression of markers identified in Aim 1, which may be specifically altered in response to GATA-6 down-regulation. We expect that once these experiments are complete, they will provide us with novel insights about the molecular mechanisms contributing to vascular remodeling in PAH.
Treatment with inhaled NO (iNO) is often effective in improving outcomes of many newborns with PPHN; however, some infants fail to respond to iNO, and require ECMO therapy. PPHN associated with poor responsiveness to iNO is often seen in the setting of hypertensive vascular remodeling and impaired vascular growth, e.g., in patients with congenital diaphragmatic hernia. In this setting, there is a critical need for therapies that can prevent vascular remodeling and stimulate angiogenesis. Partial ligation of the ductus arteriosus (DA) in utero in late gestation fetal sheep provides a useful animal model for studying the pathogenesis and treatment of PPHN. This animal model is characterized by iNO unresponsiveness, vascular remodeling and impaired angiogenesis, closely mimicking clinical PPHN and, to date, we have demonstrated altered endothelial cell (PAEC) and smooth muscle cell (PASMC) phenotypes that persist in vitro. The uniqueness of this experimental model, as well as the presence of in vitro PAEC and PASMC phenotypes, makes it innovative and crucial for studying mechanisms responsible for hypertensive vascular remodeling and impaired angiogenesis in PPHN. ET-1 and rho-kinase (ROCK) are present in the fetal lung, and contribute to high PVR, which is necessary for diversion of blood
from the lungs during fetal life. ET-1 and ROCK are important mediators of vascular remodeling and increased tone in adult models of experimental pulmonary hypertension; however, their role in neonatal PPHN is less well defined. We recently reported a role for ROCK2 in regulating angiogenesis in the developing lung and the finding that high ROCK activity contributes to impaired angiogenesis in PPHN. While the effect of ET-1 on angiogenesis in the developing lung remains unknown, ET-1 is an important regulator of ROCK activity and many of the effects of ROCK parallel those of ET-1. Gaining a better understanding of the mechanisms of ROCK and ET-1 interactions that produce vascular remodeling and impaired angiogenesis in severe neonatal PPHN will lay the foundation for the development of novel therapies that will improve outcomes in this setting.
Michael J. Passineau, PhD
Allegheny General Hospital
Pittsburgh, Pennsylvania

Gene Therapy to Drive Endogenous Biosynthesis of Prostacyclin

The overall goal of this proposal is to design and test a gene therapy strategy for pulmonary arterial hypertension (PAH). PAH is a rare, deadly, and incurable disease with a mean survival of 2.8 years from onset of symptoms, if left untreated. Of the three classes of approved therapeutics, endothelin receptor antagonists, phosphodiesterase inhibitors, and prostacyclins, prostacyclin is the most effective therapy. However, complicated delivery systems and potential side effects associated with the present formulation of prostanoids (e.g., prostacyclin) have deterred some patients and caregivers from instituting this highly effective class of agents. The challenge to be addressed by this proposal is the need for a therapeutic regimen that allows for endogenous production of prostacyclin therapy within the patient’s own body, throughout the entire lifetime of the patient. Because prostacyclin can be produced endogenously through expression of the enzyme prostacyclin synthase (PGIS), gene therapy has previously shown proof-of-principle efficacy in animal models by enabling endogenous production of prostacyclin and reversal of experimental PAH. This proposal will build upon this concept, but will execute this strategy using newly developed gene transfer technology that obviates the viral gene delivery vectors used in prior studies. Viral vectors have been extremely useful in earlier studies, but
have limited duration of expression, and, due to host immune response, cannot affect life-long therapy nor can they be re-dosed. Our gene delivery system, which uses ultrasound-induced microbubble cavitation to allow entry of non-viral DNA vectors into cells, is thought to evade host immune responses, theoretically allowing re-dosing of the PGIS therapeutic transgene as a periodic booster throughout the entire lifespan of the patient. An additional innovation of this proposal toward achieving the field-wide goal of endogenous prostacyclin production is the choice of the salivary glands as the therapeutic biosynthesis site. The salivary glands can be accessed through a bloodless, outpatient procedure, and contain a robust endocrine secretory pathway capable of secreting transgene products into the intravascular space. The encapsulated, fixed volume of the intraductal labyrinth of the salivary glands also allows precise control of the delivery system, making ultrasound-assisted gene transfer (UAGT) far more practical and consistent than has been observed in other organs (e.g., heart or pancreas). A final innovation of this proposal is the first in vivo application of a Cox-1/PGIS fusion protein that produces dramatically higher levels of prostacyclin than PGIS alone. In summary, this project seeks to innovatively test the enticing idea of PGIS-based gene therapy for PAH, aggregating three enabling technologies: 1) a practical, re-dosable gene delivery technique; 2) a novel biosynthesis site (salivary glands) that can be accessed through an outpatient procedure; and 3) a Cox-1/PGIS fusion protein transgene that produces superior levels of therapeutic prostacyclin. The efficacy of this gene therapy strategy will be tested in a highly relevant rat model of severe PAH.
Michael York, MD  
Boston University Medical Center  
Boston, Massachusetts  

dsRNA Stimulates Toll-like Receptor-3  
and Increases Endothelin-1 Production by  
Pulmonary Artery Endothelial Cells

We propose, in this work, that dsRNA stimulates toll-like receptor [TLR]3 on endothelial cells, causing endothelin-1 [ET-1]-mediated fibrosis, vasoconstriction, and inflammation, as a mechanism of relevance in immune-mediated causes of pulmonary arterial hypertension [PAH]. Research will focus on investigating how the immune system causes endothelial cell dysfunction, a hallmark of systemic sclerosis [SSc], and how these events may lead to PAH, a frequent cause of mortality in SSc. We have shown that interferon [IFN]-regulated genes are expressed highly in SSc patients’ peripheral blood mononuclear cells and also have demonstrated that this can be replicated by nucleic acid-containing immune complexes stimulating TLRs. We have recently extended this finding to endothelial cells and hypothesize that the phenotype of SSc, particularly the vasculopathy, may be induced through extracellular dsRNA binding to a specific TLR on endothelial cells, called TLR3. TLR3 is unique among the family of TLRs in that it signals using a different adaptor protein, and also has both endosomal and cell surface expression in endothelial cells. Stimulation of endothelial cell TLR3 has been shown to be pro-inflammatory and inhibit blood vessel growth [antiangiogenesis]. Our preliminary data demonstrate that dsRNA stimulates endothelial cells via a TLR3-dependent
mechanism to produce the vasoactive and pro-fibrotic peptide, ET-1, a molecule strongly implicated in SSc pathogenesis. We also show that continuous infusion of dsRNA into mouse dermis leads to inflammation, marked up-regulation of IFN-regulated genes, increased ET-1 in the skin, lung, and serum, and increased dermal fibrosis. All of these effects are abrogated in mice deficient in TLR3 signaling. Therefore, we hypothesize that dsRNA, possibly contained in circulating immune complexes or in cellular debris, triggers TLR3 on endothelial cells, resulting in inflammation, endothelial cell dysfunction, and vasculopathy. To test our hypothesis, we will: 1) examine the role of TLR3 stimulation in pulmonary artery endothelial cell (PAEC) dysfunction and apoptosis; and 2) investigate the effects of prolonged infusion of dsRNA into mice and quantify endothelial cell activation, ET-1 production, and histologic changes.
Pulmonary hypertension (PH) is characterized by elevation in pulmonary arterial pressures leading to hypoxemia and right ventricular dysfunction. While idiopathic pulmonary arterial hypertension is rare, PH complicates and worsens the prognoses of many pulmonary and systemic diseases including chronic obstructive pulmonary disease, pulmonary fibrosis, human immunodeficiency virus, systemic sclerosis, and liver cirrhosis. We have previously found that pharmacologic inhibition of MetAP2 with fumagillin decreases pulmonary fibrosis in mice injured with bleomycin. We have also found that fumagillin prevents the development of PH in monocrotaline-injured rats. In this study, we will determine if fumagillin can attenuate both pulmonary fibrosis and PH in animal models of secondary pulmonary hypertension. The ultimate goal of our work is to uncover novel therapeutic approaches to PH.
Pulmonary hypertension in patients with congenital diaphragmatic hernia is highly lethal. Presently no definitive treatment exists for pulmonary hypertension because the pathogenic mechanisms are not fully understood. Pulmonary vascular remodeling is the central cause of pulmonary hypertension, and smooth muscle cells are a major participant in driving this process. My proposal will examine smooth muscle cell related mechanisms in pulmonary hypertension. I will explore the role of Slit3 in smooth muscle cell differentiation and migration, and I will use the Slit3 null mouse to examine the impact of Slit3 upon the developing pulmonary circulation and on pulmonary circulation physiology. The work will be accomplished through three aims.

Aim 1: Determine the molecular effects of Slit3 signaling on phenotype diversity, differentiation, and gene expression in pulmonary artery smooth muscle cells in vitro.

Aim 2: Characterization of the role of Slit3 upon pulmonary artery smooth muscle cell migration related to pulmonary vascular remodeling.

Aim 3: Determine the role of Slit 3 in physiologic function and structural development of the pulmonary circulation in a mouse model of congenital diaphragmatic hernia. Findings from this work may define direct therapeutic targets to treat pulmonary hypertension in patients with congenital diaphragmatic hernia. My results may also elucidate novel treatments for other forms of pulmonary hypertension.
Pulmonary hypertension (PH) is a heterogeneous syndrome with diverse etiologies. Pulmonary arterial hypertension (PAH) comprises a small subset of PH, whereas a number of more common cardiopulmonary diseases can also lead to PH.

Compared to heart failure and chronic obstructive pulmonary disease, PAH is rare. Nevertheless, because of its poor prognosis and the exceptionally high cost of recently introduced therapies, PAH has disproportionate public health and policy significance. Other costs associated with PAH and other forms of PH, from outpatient care to diagnostic testing to hospitalization, have received relatively little attention. This project aims to provide comprehensive data on the epidemiology of hospitalizations for PH in the United States, including trends in the frequency of these events, as well as predictors of adverse outcomes, readmission, and costs associated with PH admissions.

**Specific Aim 1**: To estimate the frequency of and indications for PH and PAH hospital admissions in the United States.

**Specific Aim 2**: To determine the predictors of length of stay, death during hospitalization, and hospital readmission for PH overall and subtypes.

**Specific Aim 3**: To study the determinants of costs associated with PH hospitalizations in the United States.
Michael Eric Yeager, PhD
University of Colorado School of Medicine
Aurora, Colorado

Circulating Mesenchymal Precursors in Severe PAH and the Role of Endothelin-1 in their Recruitment and Differentiation into Fibrocytes

Vascular remodeling in chronic hypoxic pulmonary artery hypertension (PAH) occurs throughout all three layers in resistance and main pulmonary arteries. Following hypoxia, fibro-proliferative change is evident in the adventitial layer and is characterized by the presence of monocyte lineage cells derived from circulating mesenchymal precursors. The vascular remodeling observed is causally related to the influx of mesenchymal precursors because: 1) depletion of these precursor cells ameliorates the vascular change, and 2) both hypoxia and endothelin-1 [ET-1] are required for neovascularization of rat pulmonary arteries by activated fibroblasts. For this proposal, it is hypothesized that circulating mesenchymal precursors are increased in severe PAH and correlate with poor prognosis. Furthermore, it is hypothesized that endothelin-1 is a critical homing signal for the recruitment and differentiation of mesenchymal precursors that drive the remodeling process. The successful conclusion of this work will result in a clearer picture of the vascular remodeling associated with monocyte infiltration and provide the potential for a novel therapeutic approach for PAH.
Background

Maladaptive hypertrophic changes in the right ventricle (RV) are the primary cause of morbidity and mortality in patients with pulmonary arterial hypertension. Although the mechanisms responsible for modulating RV hypertrophic responses to increased pulmonary arterial pressure are not well known, endothelin (ET) receptor activation has been shown to be involved in the pathophysiology of RV hypertrophy (RVH) in several animal models of RV overload, including hypoxia- and monocrotaline-induced pulmonary hypertension [1–3]. Investigations have confirmed the role of ETs as well as protein kinase C (PKC) activation in cardiac hypertrophy [1–5]. Recent studies in rats suggest that the PKC isoform PKCδ may be particularly important in modulating RV hypertrophic responses to increased RV afterload [1]. However, the effect of ET receptor antagonism on the expression and localization of PKC isoforms in RVH is not known. In this study, we sought to evaluate the effect of treatment with bosentan, an ET receptor antagonist, on development of RVH, expression of PKC isoforms, and downstream signaling pathways in the RVs and left ventricles (LVs) of rats exposed to chronic hypoxia.
Final Report

Methods
Adult male rats were placed in either a normoxic or normobaric hypoxic environment and treated daily with vehicle or 100 mg/kg bosentan. Measurements were made in the 4 groups of: right ventricular systolic pressure (RVSP), mean systemic arterial pressure, right ventricular outflow tract diameter, heart rate, pulmonary acceleration time (PAT), and velocity time integral. Stroke volume and cardiac output were calculated.

Results
RVSP was significantly higher in hypoxic vs normoxic animals after 3 weeks of exposure. (RVSP: Hypoxia: 73 ± 6 vs. Normoxia: 27 ± 2 mmHg, p<0.05). RVH was significantly higher in hypoxic rats with RV/Body Weight ratio of 1.2 ± 0.07 mg/g bodyweight compared to 0.5 ± 0.03 mg/g bodyweight in normoxic rats (p<0.05). Treatment with bosentan significantly attenuated RVH (Figure 1) (Hypoxic/vehicle: 1.2 ± 0.07; Hypoxic/bosentan: 1.0 ± 0.05 mg/g body weight, p<0.05). Bosentan had no significant affect on RVSP. There was no significant change in mean carotid arterial pressure or heart rate among the four groups.

Transthoracic echocardiogram showed decreased PAT associated with hypoxia, consistent with increased RVSP. A trend was noted toward decreased RV stroke index in the hypoxic animals that was attenuated in the bosentan-treated animals. Further experiments are required to increase the statistical power to obtain statistical significance.

Exposure to hypoxia increased the expression of PKCδ in the RV with no significant change in expression of PKC isoforms α, β2, or ε. In the LV, hypoxia exposure did not alter the expression levels of any PKC isoform. Treatment with bosentan significantly attenuated the increase in PKCδ expression in the RVs of the hypoxic animals. Subcellular fractionation of right ventricular PKCδ expression revealed an increase in cytosolic
fraction of PKCδ in hypoxic animals. No significant differences were found among the 4 groups in RV membranous PKCδ, PKCε expression, or expression of PKCδ in the LVs.

We observed no significant difference in the expression and phosphorylation of p38, or p42/44 mean arterial pressure (MAP) kinases in the RVs or LVs of animals exposed to hypoxia compared to normoxia. These pathways have been associated with downstream PKC signaling in the ventricles.

Hypoxia was associated with an increase in procollagen expression in the RV, with no change in the LV. Treatment with bosentan significantly attenuated the expression of procollagen in the RV. In contrast, we saw a decreased expression of collagen 1 and fibronectin in the RVs of the hypoxic animals. The expression of collagen 1 and fibronectin was restored upon treatment with bosentan.

**Conclusion**

Inhibition of right ventricular hypertrophic responses in rats exposed to chronic hypoxia with the ET receptor antagonist, bosentan, is associated with decreased expression of PKCδ. Hypoxia is associated with increased expression of procollagen 1 and decreased expression of collagen 1 in the RV. Bosentan treatment restores procollagen 1 and collagen 1 expression to levels similar to those found in normoxia. Maladaptive RV hypertrophic effects seen in hypoxic pulmonary hypertension are attenuated by bosentan and may be mediated via PKCδ.
Figure 1. RVSP measurements from rats exposed to normoxia or hypoxia and treated with vehicle or 100 mg/kg/d bosentan. *p<0.05 compared to normoxia/vehicle. N=15, Mean ± SEM.

References


Background

G-protein coupled receptor (GPCR) signaling plays an important role in regulation of pathologic processes involved in cardiovascular diseases. APJ is a GPCR with a 31% homology to the angiotensin II type 1 receptor. Its ligand apelin is a potent circulating inotrope and has vasodilatory properties that are at least in part mediated by nitric oxide (NO). Significant modulation of apelin and APJ expression occurs in both animal models and patients with pulmonary arterial hypertension (PAH), suggesting a yet to be defined role of apelin in the pulmonary vasculature.

Aims

To characterize the role of apelin-APJ signaling in the vasculature, with particular emphasis on the pulmonary circulation.

Results

We found that young (<6 months), APJ-deficient mice have normal pulmonary artery pressures as measured by right ventricular (RV) systolic pressure (RVSP). However, when we assessed these mice at an older age (>6 months), they developed significant pulmonary hypertension (PH; P=0.001). The APJ-deficient mice also demonstrated elevated RV/left ventricular (LV) ratio, demonstrating RV hypertrophy (P=0.02).
We subjected apelin-deficient mice and their wildtype littermates to hypoxia for two weeks. The apelin-deficient mice developed greater increase in RVSP compared to their wildtype littermates (33.23 mmHg vs. 27.67 mmHg, P=0.016, n=6 in each group).

We assessed the expression level of endothelial NO synthase (eNOS) in the lungs of the apelin-deficient and wildtype mice via real-time quantitative PCR analysis. We found that there was a 70% decrease in the transcript level of eNOS in the lungs of apelin-deficient mice (P=0.001). (Figure 1).

We found that the apelin-deficient mice have a marked decrease in their serum NO levels compared to the wildtype (P=0.02). This is consistent with the finding that eNOS expression in the apelin-deficient mice is lower compared to the wildtype.

To determine if apelin signaling is regulated by other mediators of PH, we determined whether the expression of apelin is regulated by bone morphogenic protein (BMP)2. BMP signaling is disrupted in certain cases of familial PAH. We found that apelin expression is increased by two-fold in pulmonary artery microvascular endothelial cells stimulated with BMP2.

To address a potential mechanism by which apelin may be protective against PH, we assessed its potential protection against endothelial cell apoptosis. We found that human umbilical endothelial cells (HUVECs) were protected against apoptosis driven by serum starvation by 23% in the presence of apelin.

We evaluated the serum levels of apelin in patients with PAH by ELISA assay for apelin-12 (n= 32 patients, n=10 healthy controls). We found that the serum level of apelin was significantly decreased in patients with PAH (P=0.05). This finding is consistent with our findings in animals, where apelin levels are markedly downregulated in rodents with induced PH secondary to either hypoxia or monocrotaline treatment.
We have evaluated the transcript levels of eNOS in pulmonary artery endothelial cells (PAECs) in response to downregulation of apelin expression. We found that inhibition of apelin expression led to decreased transcript levels of eNOS mRNA, as well as decreased expression of eNOS protein.

Micro-CT of the lungs demonstrated vascular abnormalities in the apelin KO mice including pruning of smaller vessels and increased tortuosity of the larger vessels consistent with other animal models of PH.

Conclusion

- APJ-deficient mice demonstrate increased pulmonary artery pressures.
- Apelin-deficient mice
  - Demonstrate worsening PH compared to wildtype mice when subjected to hypoxia.
  - Have decreased expression of eNOS in the lungs.
  - Have decreased levels of serum NO.
- Apelin
  - Is upregulated by BMP.
  - Signaling inhibits endothelial cell apoptosis.
- Serum apelin levels are decreased in patients with PAH.
- Downregulation of apelin expression in PAEC leads to decreased eNOS expression.
- Micro-CT of apelin-deficient mice demonstrate pruning of smaller pulmonary vessels.

These data demonstrate an emerging importance of the apelin-APJ signaling in PH, which is at least in part through the downregulation of eNOS expression, and demonstrate its potential as a therapeutic target.
Figure 1. Apelin-deficient mice showed a 70% decrease in the transcript level of eNOS in the lungs ($P=0.001$).
Background

Our original proposal was to examine the effect of a BMPR2 mutation on the endothelin (ET)-1 cascade using lung endothelial cells (ECs) and pulmonary artery smooth muscle cells cultured from a unique ROSA26-tet-BMPR2<sup>R899X</sup> transgenic (ROSA26-transgenic) mouse model of familial pulmonary arterial hypertension (FPAH). Our collaborator, Dr. James West, moved to Vanderbilt in October, 2007 and brought with him breeding pairs of ROSA26-rtTA and ROSA26-transgenic mice. However, we have been unable to breed mice in sufficient numbers to carry out these studies.

A recent paper by Dr. West, using a mouse model of PAH with a BMPR2 mutation, demonstrated large numbers of macrophages and T cells in the adventitial layer of the pulmonary arteries [1]. A similar infiltration of cells has been found in the plexiform lesions in idiopathic PAH [2]. Therefore, we modified our research plan and utilized cultured bone marrow-derived macrophages (BMMs, precursors of tissue macrophages) for the following studies.

Under several pathological conditions, ET-1 expression, ET-1 secretion, and ET-1 receptor expression are increased in lung macrophages [3,4]. Whether the ET-1 cascade is altered in lung macrophages hyper-expressing a BMPR2 mutation is not known. We used cultured BMMs isolated from ROSA26-transgenic and ROSA26-rtTA mice to test our hypothesis that BMPR2 mutation alters the macrophage ET-1 cascade.
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Aims

Specific Aim 1: Determine expression of ET-1, ET type A receptor (ETA-R), and ET type B receptor (ETB-R) in FPAH (with BMPR2 mutation) and control human lung tissue.

Specific Aim 2: Determine the expression of ET-1, ET-1 receptors, and ET-converting enzyme (ECE) in cultured BMMs from the ROSA26-transgenic mouse model of FPAH and ROSA26- rtTA mouse [control].

Results

Localization of ET-1 in human lung macrophages was similar in controls and FPAH lungs. However, immunohistochemical and co-localization studies revealed that ETA and ETB expression was decreased in FPAH lung macrophages vs. control cells.

ETA-R and ETB-R gene expression is significantly greater in control macrophages vs. hypertensive mouse model macrophages at rest. On activation with LPS, ETA gene expression is decreased significantly compared to controls, whereas ETB gene expression remains unchanged.

At rest, ppET-1 gene expression is low in control and hypertensive macrophages. Following LPS stimulation, expression of ppET-1 gene is increased 6-fold and 10-fold over baseline in control and hypertensive macrophages, respectively.

At rest, relative expression of the ECE gene is similar in cultured control and hypertensive macrophages. Following LPS stimulation, ECE gene expression decreases significantly in both groups.

At rest, ET-1 secretion into the culture media is negligible. Levels of ET-1 in the supernatant increases in cells from both groups following activation with LPS but is statistically higher in the hypertensive group compared to controls.
To examine whether ETA-R and ETB-R contribute to the increased levels of ET-1 in culture supernatants, we utilized ETA-R and ETB-R inhibitors in control cells. At rest, there was no change in ET-1 secretion in the presence of ET receptor inhibitors. However, following LPS activation, ETA-R and ETB-R inhibitors (BQ-123 and BQ-788, respectively), in combination, significantly increased ET-1 in the culture medium (Figure 1). The increase in ET-1 in the presence of the ETB-R inhibitor, while not significant, suggests that this receptor is mainly responsible.

Conclusion

In summary, our results demonstrate that ET-1, ETA and ETB receptors are expressed in macrophages in human lung and expression of both ETA and ETB is down-regulated in FPAH lungs with a BMPR2 mutation. The findings for ETA and ETB can be recapitulated in a mouse model of FPAH using BMMs. In addition, the low levels of ETA expression in the hypertensive cells are further decreased upon activation: ETB expression is unchanged following activation. Our findings also demonstrate an increase in ET-1 gene expression and ET-1 levels in the supernatant in control and hypertensive cells following activation. The striking reduction in ECE gene expression in the hypertensive cells in the face of an increase in ET-1 in the media is perhaps explained by our finding of reduced ETA and ETB expression on these cells. We conclude that in FPAH, mutated BMPR2 down-regulates ET receptors and ECE of lung monocytes/macrophages. In lung macrophages with BMPR2 mutation, ET-1 may have paracrine influence due to altered ET-1 cascade.
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References


Figure 1. Effect of ETA and ETB receptor antagonists (BQ-123 and BQ-788, respectively) on ET-1 secretion in control cultured macrophages from mouse bone marrow. In presence of both inhibitors, ET-1 in culture media is significantly increased in activated cells (*P < 0.05). Data expressed as means ± SE. n = 4.
Ronald J. Oudiz, MD, FACP, FACC, FCCP is Professor of Medicine, David Geffen School of Medicine at UCLA and is the Director of the Pulmonary Hypertension Center and Faculty Cardiologist at the Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center in Southern California. Dr. Oudiz received his medical school training at the University of Southern California in Los Angeles, his Internal Medicine training at the University of California, San Diego, and his training in Cardiovascular Diseases at Harbor-UCLA Medical Center in Torrance, CA. He is board-certified in Internal Medicine and Cardiovascular Diseases. Dr. Oudiz is a past holder of scientific research awards from the American Heart Association and the National Institutes of Health. He has authored several papers in pulmonary hypertension and has presented his research at national and international seminars. Dr. Oudiz is the immediate past Editor-in-Chief of the scientific publication Advances in Pulmonary Hypertension. He has participated in several trials of innovative medical treatments for pulmonary hypertension (PH), many of which are still ongoing. Dr. Oudiz’s recent focus has been to describe the physiologic abnormalities that are caused by PH using measurements of lung gas exchange during exercise, and to study exercise rehabilitation as a treatment modality for patients with PH.
Biography

Jocelyn Dupuis, MD, PhD
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Jocelyn Dupuis, MD, PhD, is Associate Professor of Medicine in the Department of Medicine at the University of Montreal. He is a Cardiologist at the Montreal Heart Institute and National Researcher of the Quebec Health Research Funds. He serves as President of the Pharmacology Committee at the Montreal Heart Institute in Montreal.

Dr. Dupuis earned a medical degree from the University of Montreal and a PhD in Experimental Medicine from McGill University. He completed an internship in Medicine at McGill University and post doctoral work in Internal Medicine and Cardiology at Sherbrooke University.

Dr Dupuis is a member of the editorial boards of the Journal of the American College of Cardiology, the American Journal of Pathology, and the Canadian Journal of Cardiology. He has been published in numerous peer-reviewed journals, including Circulation, Journal of the American College of Cardiology, Journal of Nuclear Medicine, Cardiovascular Research, and American Journal of Respiratory and Critical Care Medicine.
Dr. Harrison [Hap] Farber is a Professor in the Department of Medicine and the Director of the Pulmonary Hypertension Center at Boston University.

He has focused on research into pulmonary arterial hypertension (PAH) and the clinical care of PAH patients for approximately 20 years. Dr. Farber has received numerous grants (both basic science and clinical) and has an extensive publication record in this area, including articles in peer-reviewed journals such as Circulation, New England Journal of Medicine, and Chest.

Dr. Farber serves on many panels for the development of clinical recommendations in PAH, has participated in large multicenter clinical trials, and is on the Steering Committee of the REVEAL Registry (Registry to Evaluate Early and Long Term PAH Disease Management), the largest registry of PAH patients ever created. His research interests include endothelial cell biology, in particular, the response of the pulmonary vasculature to injury.

After earning a medical degree at George Washington University School of Medicine, Dr. Farber completed an internship and residency at the Medical College of Virginia and a fellowship at Boston University.
Dr. Adaani Frost is Professor of Medicine in the Pulmonary and Critical Care Section of Baylor College of Medicine, Houston, Texas. She undertook her postgraduate training in pulmonary and critical care including a fellowship in lung transplantation in the Toronto Hospital System and McGill University. She was Medical Director of the Lung Transplant Program at both the Methodist Hospital and St. Luke’s Episcopal Hospital from 1990 to 2001 and has since developed the Pulmonary Hypertension and Advanced Lung Disease Service at Baylor. Currently, she is involved in clinical management and clinical research on patients with end stage lung disease, predominantly in pulmonary hypertension, and pulmonary fibrosis. Dr. Frost was on the Scientific Advisory Council of the Pulmonary Hypertension Association until 2009, is on the steering committee of REVEAL [a US-based registry of more than 3500 pulmonary hypertensive patients], has authored numerous papers on pulmonary hypertension, and is a participant in multiple new and ongoing studies in the treatment of pulmonary hypertension.
Dr. Mayes graduated from Eastern Virginia Medical School and completed her Internal Medicine training and Rheumatology fellowship at the Cleveland Clinic. She received a Master’s in Public Health (MPH) in Epidemiology from the University of Michigan School of Public Health. She joined the University of Texas – Houston Medical School faculty in 2002 and subsequently established the Scleroderma Clinic. Dr. Mayes is the recipient of many distinctions, awards and grants for the study and treatment of scleroderma. She is the author of over 100 published manuscripts, 19 reviews, 5 book chapters and 1 full length book. Her clinical interests include the treatment of scleroderma and its multiple complications. She participates in several multi-center, national trials of new agents for this disease. Her research interests include the identification of susceptibility genes and disease severity genes in scleroderma and related autoimmune diseases. She is currently the Principal Investigator of the NIH/NIAMS funded ‘Two-Stage Genome-Wide Association Study in Scleroderma’ that has the dual objectives to identify genes that influence disease susceptibility and severity, as well as to serve as a national resource to supply genetic material to other investigators to study this disease.
Dr. Michael McGoon is Professor of Medicine and Consultant in Cardiology at the Mayo Clinic, in his hometown of Rochester, Minnesota, where he also serves as Associate Chair (Personnel) of the Division of Cardiovascular Diseases. Dr. McGoon attended medical school at the Johns Hopkins School of Medicine, after which he completed an internship and one year of residency at Johns Hopkins, before transferring to Mayo for his final year of residency and cardiology training. He has remained on staff at Mayo since then. During his cardiology fellowship, he developed an interest in the hemodynamic and physiologic effects of vasodilators and other medications in patients with pulmonary hypertension under the mentorship of Dr. Ronald Vlietstra, Dr. Valentin Fuster and Dr. Paul Vanhoutte. That interest led to participation in multiple clinical trials of pulmonary vascular medications, starting with prostacyclin, and continuing to the present time. Dr. McGoon is board certified in Internal Medicine and in Cardiology. He was Director of the Pulmonary Hypertension Clinic at Mayo since he initiated it in 1996 until 2007. He has been a member of the Scientific Advisory Board (now Scientific Leadership Council) of the Pulmonary Hypertension Association (PHA) since 1994, serving as its Chair from 2002 to 2004. He was Chair of the Board of Trustees of the PHA (2006-2008), and currently chairs the PHA Development Committee. He also serves as chair of the Steering Committee of the Registry to Evaluate Early and Long-term Pulmonary Arterial Hypertension Disease Management (REVEAL).
Harold I. Palevsky, MD
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Dr. Palevsky earned a medical degree from the Medical College of Virginia. He completed an internship and residency in internal medicine, and a fellowship in pulmonary and critical care medicine at the Hospital of the University of Pennsylvania, where he worked with Alfred P. Fishman, MD.

His clinical and research interests include unexplained dyspnea, lung transplant evaluation, pulmonary vascular disease, pulmonary hypertension, and thromboembolic disease. Dr. Palevsky has been published in numerous peer-reviewed journals, including the Annals of Internal Medicine, JAMA, and Circulation. He has been recognized as one of Philadelphia’s “Top Docs” and is included in national lists such as “The Best Doctors in America” and the “Guide to America’s Top Physicians.”
Biography

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Ivan M. Robbins, MD, is an Associate Professor of Medicine in the Department of Medicine, Division of Allergy, Pulmonary, and Critical Care Medicine, at the Vanderbilt University School of Medicine. He is also the Director of the Pulmonary Vascular Center at Vanderbilt University Medical Center.

Dr. Robbins earned a medical degree from Case Western University School of Medicine, completed an internship and residency at Metrohealth Medical Center, and pursued a Pulmonary and Critical Care fellowship from Vanderbilt University School of Medicine.

An internationally recognized expert in the field of pulmonary vascular disease, Dr. Robbins’ research interests include the mechanisms of action of epoprostenol and the role of oxidant stress in pulmonary arterial hypertension. He has been published in numerous peer-reviewed journals, including Circulation, American Journal of Respiratory and Critical Care Medicine, and the Journal of the American College of Cardiology.
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Biography

Richard M. Silver, MD
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Dr. Richard Silver serves as Director of the Division of Rheumatology & Immunology at the Medical University of South Carolina (MUSC). He was born in Tennessee and graduated from the University of Tennessee – Knoxville. After graduating from Vanderbilt University School of Medicine in 1975, Dr. Silver completed training in Internal Medicine at the University of North Carolina-Chapel Hill, and then in Rheumatology at London’s Northwick Park Hospital and at the University of California-San Diego. He joined the faculty at MUSC in 1981, where currently he is Professor of Medicine and Pediatrics and is the Director of the Division of Rheumatology and Immunology. In 2007, MUSC’s Board of Trustees named him a “Master Teacher” and bestowed the University’s highest academic recognition, Distinguished University Professor. Also in 2007, the Scleroderma Foundation named him their “Doctor of the Year.” Dr. Silver’s major research interest is Interstitial Lung Disease associated with Systemic Sclerosis.
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