Large Vessel Stiffening and Pulmonary Hypertension: Contribution of Extracellular Proteins and Inflammation

K. R. Stenmark

University of Colorado Denver, CO 80262, USA

RV Failure is the Proximate Cause of Death in Patients With Pulmonary Hypertension

Reproduced from Ghio S et al. JACC, 2001

Pulmonary Circulation: Questions

- Is there evidence for central vascular stiffening in PH?
- What are the potential cellular mechanisms contributing to stiffening of the pulmonary artery?
- Should/can anything be done to directly reduce proximal vascular stiffening in PH?

Increased resistance to blood flow

Increased afterload

Right Heart Failure

Increased stiffness of pulmonary artery

Death

Note: “stiffness” is the inverse of “compliance”

Historical understanding: resistance determines afterload. Drugs lower resistance, but have not adequately improved outcomes. It’s now known that resistance and compliance determine afterload.
Pulmonary Hypertensive Vessels Enlarge and Stiffen.

- Normotensive PA
- Hypertensive PA

D = 1.6 cm
D = 2.5 cm

Artery Wall At Systole
Wall Deformation Over Cardiac Cycle

Measured by MRI (Age, Gender and BSA Matched)

Significant Increases in Impedance are Observed in Patients with PAH

- Significant elevation in modulus of impedance for the first three harmonics in patients with PH.
- \( Z_0 \): resistance, \( Z_1, Z_2 \): oscillatory function / capacitance

Hunter et al., Am J H., 2008

PAH-Associated Vascular Remodeling in Human

Normal PA
Hypertensive PA

Collagen
Elastin fragmentation
Collagen accumulation

2-Photon Second Harmonic Generation Microscopy: Elastin; Collagen

Physiologic Stiffness is Increased in Patients with PAH

Circumferential Stress

PA Stiffening in Severe PH

HEALTHY
HYPERTENSIVE
Can Animal Models be Used to Study Proximal Vascular Stiffening in Pulmonary Hypertension?

Chronic Hypoxia Results in Stiffening of the Extralobar Pulmonary Arteries in Mice and Rats

Marked Increases in Resistance and Stiffening in Chronically Hypoxic Neonatal Calves

<table>
<thead>
<tr>
<th></th>
<th>Indices of Ventricular Resistance</th>
<th>Indices of Pulmonary Vascular Stiffening</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak Systolic Pressure (mm Hg)</td>
<td>Mean PAP (mm Hg)</td>
</tr>
<tr>
<td>Neocrotal (2 wk-old) Calves</td>
<td>Control (n=12)</td>
<td>31.8±3</td>
</tr>
<tr>
<td></td>
<td>Moderate PH (n=6)</td>
<td>59.4±7.5</td>
</tr>
<tr>
<td></td>
<td>Severe PH (n=8)</td>
<td>191.3±17.3</td>
</tr>
</tbody>
</table>

Interim Summary: PH-Associated Pulmonary Vascular Remodeling in Human

- Significantly elevated stiffness
- Early collagen engagement
- Significant anisotropy (different longitudinal vs. circumferential mechanics)
- Loss of elastin-dependent mechanics
- Near total loss of PA capacitive function

Matrix Remodeling in PH

FUNCTIONAL:
- Elastic fragmentation
- Collagen accumulation

STRUCTURAL:
- Elastin fragmentation
- Collagen accumulation

Indices of Pulmonary Vascular Stiffening

- MPA Stiffness (mm KPa)
- Pulse Pressure (mm Hg)
- Mean PAP (mm Hg)
- Total Pulmonary Resistance (mm Hg L/min)

Indices of Ventricular Resistance

- Peak Systolic Pressure (mm Hg)
- Mean PAP (mm Hg)
- Total Pulmonary Resistance (mm Hg L/min)
- Pulse Pressure (mm Hg)
- MPAP Strain (mm KPa)
Pulmonary Circulation: Questions

• Is there evidence for central vascular stiffening in pulmonary hypertension?

• What are the potential cellular mechanisms contributing to stiffening of the pulmonary artery?

• Should/can anything be done to directly reduce proximal vascular stiffening in PH?

Hypothesis:
Large Vessel Stiffening is Due to Accumulation and Activation of Mononuclear Cells

Humans with PAH:
Accumulation of Monocyte/Macrophage in Pulmonary Artery Media, Neointima, and Adventitia

• PA media normally contains “resident” immunomodulatory leukocytic cells;
• in PAH, numbers of these cells within vascular media markedly increase.

Calf Model Exhibits Greater Similarities to Human PAH than Rodent Models

Accumulation of inflammatory cells in PA Media, Adventitia, (and Neointima); Elastin fragmentation is observed

Influx of inflammatory cells in PA Adventitia only
Extracellular Matrix (ECM) Derived Fragments Can Drive a Vascular Inflammatory Response

ECM peptides with immune / inflammatory effects:
- Elastin
- Collagen (I and IV)
- Fibronectin
- Laminin
- Thrombospondin
- Hyaluron

Vascular Inflammation, Elastin Fragmentation, and Vascular Stiffening

Activated Macrophages
Degradation of Elastin
Elastin Fragments ("Elastokines")

- Chemotactic for leukocytes
- Pro-Mitogenic for VSMCs & Fibs
- Anti-Apoptotic
- Pro-Angiogenic

Chronic Inflammation
Vascular Remodeling

Expression of Pro-Inflammatory Mediators, Pro-Fibrotic Markers, and Proteolytic Enzymes is Augmented in Vascular Media of Hypoxic Hypertensive Calves (Hx)

Bone Marrow-Derived Macrophages (BMDMs) – but not SMCs –Respond to Elastin Peptide Treatment by Upregulating Pro-Inflammatory Mediators
Bone Marrow-Derived Macrophages (BMDMs) – but not SMCs – Responded to Elastin Peptide Treatment by Upregulating Pro-Fibrotic Markers

Ccl12 (SDF-1)  
Ssp1 (Osteopontin)  
Tnc (Tenascin-C)  
Col3a1 (Collagen III)  
Col1a1 (Collagen I)  
Thbs1 (Thrombospondin-1)

Relative Expression to HPRT Relative Expression to HPRT

Interim Summary:

• Normal conduit PA media (human and bovine, but not rodent) contains “resident” inflammatory/immunomodulatory leukocytic cells.

• In pulmonary hypertension, increased accumulation of numbers of these leukocytic cells is observed.

• Elastin peptides induce activation of naïve macrophages (BMDMs): upregulated mRNA expression of pro-inflammatory and pro-fibrotic markers.

Pulmonary Circulation: Questions

• Is there evidence for central vascular stiffening in pulmonary hypertension?

• What are the potential cellular mechanisms contributing to stiffening of the pulmonary artery?

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Compliance is Fundamental to PH

- Low compliance is the leading independent predictor of mortality
- Compliance correlates directly with exercise capacity and quality of life
- Low compliance accounts for up to 50% of RV afterload
- Low compliance stimulates progression of the disease
- Pulmonary vascular stiffness is increased 25- to 30-fold
- etc...

Potential Mechanisms of HPF-Induced Perpetuation of Distal PA Remodeling In PH

- HPF
- Normal human (anti-e-SMA stain)
- Normal calf (H&E stain)
- Human PH
- Hypoxia-induced calf PH
- Endothelium
- Fibroblasts
- Vascular SMCs
- SMC hypertrophy
- Fibs–Myofibs differentiation
- Membranous
- EndoMT
- Mural

Therapies Aimed at Reducing Vascular Stiffening

- Renin Angiotensin Aldosterone System (RAAS) blockers
- AGE – cross-link breakers (alagebrium)
- Statins
- Anti-Inflammatory agents
- Elafin (serine protease inhibitor)

Aria CV’s Device Restores Compliance to PA

- Implanted like a pacemaker
- Mimics function of elastic pulmonary artery
- Balloon collapses during systole & expands during diastole
- Acts like a passive IABP
- By restoring compliance, it allows more blood to flow for less heart work

Bench: Hemodynamic Benefits

- Lower systolic pressure
- Reduced RV work & wall stress
- Increases SV
- Higher diastolic pressure
- Increases blood flow
- Reduction of pulse pressure
- Breaks the disease-stimulating feedback loop
- Increase in exercise capacity & QOL
- Decrease in mortality likely

- Compliance increased by 0.51 ml/mmHg (in this example)

- Pressure in Pulmonary Artery
- Systole
- Diastole

- Device
- Hemodynamic Benefits
- Expected Patient Effects
Do we want to say that it functions similar to an IABP?

Stick in slide with benchtop data in it.

Karl Vollmers, 6/17/2013
**Bovine Studies**

- Device efficacy studies using high altitude cattle
- Brisket disease = RHF due to PH caused by chronic hypoxia
- Histology of the pulmonary vessels resembles vessels of PAH patients

Device increased Pulmonary Vascular Compliance by 1.3 ml/mmHg (~47% increase)

**Device in vivo**

**Implications for PH Patients: Mayo Clinic Study**

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Compliance (ml/mmHg)</th>
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<tbody>
<tr>
<td>Healthy</td>
<td>6–10</td>
</tr>
<tr>
<td>Q1</td>
<td>2.0–3.77</td>
</tr>
<tr>
<td>Q2</td>
<td>1.26–2.0</td>
</tr>
<tr>
<td>Q3</td>
<td>0.82–1.25</td>
</tr>
<tr>
<td>Q4</td>
<td>0.4–0.81</td>
</tr>
</tbody>
</table>

- An increase of compliance by 1.3 ml/mmHg (as in Bovine Study 3) would move patients up two quartiles.
- Good chance of Survival Benefit

**Compliance is Fundamental to PH**

Relationship of Capacitance and Mortality in Patients with iPAH (JACC 2006, McGoon, et al)

Conclusion:
Pulmonary Hypertension is a Disease of Disordered Coupling of the RV to the Pulmonary Circulation, Where Large Vessel Stiffening is Critical

High Afterload → Right Heart Failure.

Elevated mPAP, pulse pressure, and disturbed flow patterns → High Afterload → Right Heart Failure.

Large Vessel Stiffening, via Effects on Pulse Wave Intensity, Modulates Distal Vessel Structure and Function

Mimetic Circulatory System

High Pulsatility Flow (HPF) Stimulates Bovine Distal PA-EC Inflammation
Ventricular-Arterial Coupling in the Pulmonary Circulation

Endothelium:
- Endothelial dysfunction
- Increased permeability

Extrinsic Influences: NaCl, Lipids, AngII, Sympathetic Neurohormones, Shear Stress, Increased Luminal Diameter

Potential Mechanisms Contributing to Vascular Stiffening

Endothelium:
- Endothelial dysfunction
- Increased permeability

Media:
- ↑ Collagen, VSMCs, AGE's, MMPs, Mφ?
- ↓ Elastin, (elastases↑)

Adventitia:
- ↑ Collagen, Fibroblasts
- ↑ Mφ, T-cells

Intima:
- ↑ Collagen, VSMCs, Leukocytes (Mφ), MMPs, AGE’s, TGF-β, etc etc.
- ↑ Elastin

Extrinsic Influences: NaCl, Lipids, AngII, Sympathetic Neurohormones, Shear Stress, Increased Luminal Diameter

In Vivo Depletion of Macrophages via Clodronate-Liposomes Results in Attenuation of Hypoxia-Induced Fibrosis and Vascular Remodeling in Rats

Hypothesis

In pulmonary hypertension, vascular remodeling and stiffening are the result of augmented Accumulation and Activation of Inflammatory / Immunomodulatory cells within the media of large PAs.
Potential Mechanisms Contributing to Vascular Stiffening

Endothelium:
• Endothelial dysfunction
• Increased permeability

Media:
• Collagen, VSMCs, AGE’s, MMPs, MΦ?
• ↓ Elastin, (elastases↑)

Adventitia:
• Collagen, Fibroblasts
• MΦ, T-cells

Intima:
• Collagen, VSMCs, Leukocytes (MΦ), MMPs, AGE’s, TGF-β, etc etc.
• ↓ Elastin

Extrinsic Influences: NaCl, Lipids, AngII, Sympathetic Neurohormones, Shear Stress, Increased Luminal Diameter

Hypothesis
Elastin peptides, generated in the setting of PH in the media of large elastic PAs, perpetuate pro-inflammatory and pro-fibrogenic gene programs in monocytes/macrophages.

Elafin Treatment Reverses Established Severe PH in Rats

*P <0.05, One-way ANOVA with Dunnett’s Multiple Comparison

N. Nickel and M. Rabinovitch unpublished