The pathophysiology of pulmonary hypertension (PH) is multifactorial, complex, and incompletely understood. However, it is known that abnormal mechanical forces within the pulmonary vasculature participate in the disease process. The pulmonary vasculature is continually exposed to hemodynamic forces that include: (1) shear stress, the tangential friction force acting on the vessel wall due to blood flow; (2) hydrostatic pressure, the perpendicular force acting on the vascular wall; and (3) cyclic strain, the circumferential stretch of the vessel wall. Mechanosensors on pulmonary vascular endothelial cells detect these forces and transduce them into biochemical signals that trigger vascular responses. Among the various force-induced signaling molecules, nitric oxide (NO), reactive oxygen species (ROS), and endothelin-1 (ET-1) have been implicated in vascular health and disease. For example, increases in physiologic shear stress associated with increased cardiac output result in induction of NO production with decreased ROS and ET-1, facilitating pulmonary vasodilation and increased flow. However, the pathologic pulmonary vasculature may induce supraphysiologic levels of shear stress, pressure, and cyclic strain resulting in decreased NO with increased ROS and ET-1. Thus, abnormal hemodynamic forces develop in and participate in the disease progression of most forms of pulmonary vascular disease (PVD). However, the influence of hemodynamic forces in the pathobiology of PVD is most clearly demonstrated in patients with PH secondary to congenital heart disease (CHD). PH SECONDARY TO CHD CHD remains one of the most common worldwide causes of PVD, and represents 45% to 55% of all pediatric PVD. In these patients, structural cardiac abnormalities result in increased flow within the pulmonary vasculature—with or without a direct pressure stimulus from the systemic ventricle—that in turn cause well-described progressive histopathologic changes within the pulmonary circulation. Classification of PVD associated with CHD belies the complexity and varying physiology of predisposing cardiac lesions—from the classic example of unrestrictive ventricular septal defect (VSD) to complex single-ventricle lesions. The natural history of PVD associated with systemic-to-pulmonary shunt reveals the differential, or perhaps incremental, effects of increased pulmonary blood flow and increased pulmonary arterial pressure. In patients with increased blood flow alone—pre-tricuspid valve lesions such as atrial septal defects (ASDs)—the development of PVD is uncommon and presents late, among 5% to 15% of patients by the fourth decade of life. In stark contrast, in patients with increased blood flow and a direct pressure stimulus from the systemic ventricle—post-tricuspid lesions such as unrestrictive VSDs or truncus arteriosus—the development of PVD is common, and develops early in life. The progression of PVD in these lesions reflects the differing hemodynamic insults to the pulmonary vasculature. In addition, genetic predispositions and/or differences in oxygen tension delivered to the pulmonary vasculature likely participate in disease progression, and represent an important, yet poorly understood area of investigation. A summary of the risk of developing PH SECONDARY TO CHD

Key Words—congenital heart disease, cyclic strain, hydrostatic pressure, pulmonary vascular disease, shear stress

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Figure 1: Biomechanical Forces Regulate Vessel Tone. Vascular tone is regulated by the opposing effects of vasodilators and vasoconstrictors that are predominantly produced by the vascular endothelium. These bioactive factors are heavily regulated by biomechanical forces such that laminar SS (LSS) stimulates factors that enhance vasodilation while cyclic stretch (CS) enhances vasoconstriction.
irreversible PVD with different lesions associated with increased pulmonary blood flow and the age of development is described in Table 1.

Thus, the investigation of the effect of specific physiologic and pathophysiologic hemodynamic forces on the pulmonary vasculature may lead to targeted therapeutic approaches for PVD secondary to CHD, as well as inform other types of PVD, in which abnormal mechanical forces participate in disease progression. Using endothelial cell monolayers, a growing body of in vitro literature informs the effect of different types and magnitudes of biomechanical forces on endothelial cell function. These data are summarized in the following two paragraphs.

**REGULATION OF ENDOTHELIAL VASOACTIVE FACTORS BY BIOMECHANICAL FORCES: IN VITRO STUDIES**

**Vasodilators: NO, Prostacyclin, Endothelium-derived Hyperpolarizing Factor**

Nitric oxide is a vasorelaxant produced by NO synthase isoforms converting L-arginine to citrulline. In the blood vessels, NO is synthesized in the endothelial cells (ECs) and diffuses to the adjacent smooth muscle cells (SMCs), where it activates soluble guanylate cyclases (sGC). This leads to activation of cGMP-dependent PKG and other effector proteins, including ion channels, ion pumps, and phosphodiesterases (PDEs). NO is also known to inhibit platelet aggregation and inhibit SMC proliferation. Physiologic laminar shear stress (SS) is well known to increase NO production via endothelial NO synthase (eNOS) phosphorylation and/or stimulating EC receptors and increasing intracellular Ca²⁺. Exposing ECs to laminar SS can also suppress ROS levels. Importantly, exposing ECs to either pathologic low or high levels of laminar SS, or irregular flow patterns, leads to higher levels of ROS and less available NO. A large body of evidence demonstrates that patients with advanced pulmonary vascular disease have decreased bioavailable NO and increased ROS production. Importantly, patients with PVD secondary to CHD also demonstrate early aberrations in NO production.

Derived from arachidonic acid within the EC, prostacyclin (PGI₂) is another vasodilator with a broad range of effects on the vasculature that is induced by flow (laminar SS). Prostacyclin binds to the prostacyclin receptors (IP), which are located on both platelets and SMCs and that leads to inhibition of platelet aggregation. Acting via GPCRs prostaglandin receptors, it induces cAMP synthesis and well-described PKA-dependent pathway of the cytoskeletal reorganization and relaxation. The effects of PGI₂ are tightly related to NO effects since PGI₂ potentiates NO release and, in turn, NO potentiates the effect of PGI₂ on SMCs.

Table 1. Risk of PVD in Differing Lesions Associated With CHD and Increased Pulmonary Blood Flow. These data are adapted from reference 98.

<table>
<thead>
<tr>
<th>CHD WITH INCREASED PULMONARY BLOOD FLOW AND/OR PRESSURE</th>
<th>RISK OF PVD</th>
<th>AGE OF OCCURRENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Truncus Arteriosus</td>
<td>−100%</td>
<td>&lt;2 years</td>
</tr>
<tr>
<td>A-V Septal Defect</td>
<td>−100%</td>
<td>2 years</td>
</tr>
<tr>
<td>Transportation of Great Arteries + VSD</td>
<td>−70%-100%</td>
<td>1-2 years</td>
</tr>
<tr>
<td>Patent Ductus Arteriosus</td>
<td>−15%-20%</td>
<td>&gt; 2 years</td>
</tr>
<tr>
<td>Ventricular Septal Defect</td>
<td>−15%-20%</td>
<td>&gt; 2 years</td>
</tr>
<tr>
<td>Atrial Septal Defect</td>
<td>−20%</td>
<td>&gt; 20 years</td>
</tr>
</tbody>
</table>

Defects in bold and italics represent high-flow/direct high-pressure lesions; defects in italics represent high-flow/variable direct high-pressure lesions; ASD is a high-flow lesion without a direct pressure stimulus from the systemic ventricle.

Table 1 shows the risk of PVD associated with different CHD lesions and increased pulmonary blood flow. The risk of PVD is defined as the percentage of patients with advanced PVD. The age of occurrence is defined as the number of years from the onset of CHD to the diagnosis of PVD. The table highlights the risk of PVD for different CHD lesions, ranging from 20% to 100%, and the age of occurrence, ranging from <2 years to >20 years. The data are adapted from reference 98.
of NO activity. However, physiological levels of shear stress have a negative effect on the expression of ppET-1 and ET-1-converting enzyme (ECE-1) in the EC. This downregulation of the ET-1 system depends on eNOS activation and oxidative stress. Conversely, cyclic stretch significantly upregulates prepET-1 mRNA expression in ECs. A wealth of evidence implicates ET-1 signaling in the pathophysiology of PVD secondary to CHD. For example, ET-1 mRNA and peptide expression are significantly upregulated in PH models and patients, and ET-1 levels are increased in both the plasma and lung of patients with PVD and, importantly, correlate with disease progression.

Another arachidonic acid derivative, Thromboxane A2, is secreted by platelets, inducing platelet aggregation, thrombosis, and reducing blood flow. Thromboxane A2 promotes platelet aggregation and expresses adhesives coagulant for platelets such as von Willebrand factor, fibrinecton and thrombospandin, and procoagulant factors. Thromboxane A2 exerts its biological activity through its cognate TP GPCR receptor. Thromboxane A2 receptor is known to promote cell migration and proliferation of SMCs. Thromboxane A2 is a functional antagonist of prostacyclin and balance between them supports vascular homeostasis. Interestingly, laminar SS can induce ROS by AT1R-mediated downregulation of eNOS expression, which is dependent on Akt and Erk activity.

Although these in vitro studies have been very informative, several limitations are noteworthy. For example, traditional studies of EC mechanotransduction are performed utilizing EC monolayers. Therefore, important interactions between ECs and SMCs are not captured during these studies. In addition, replicating in vivo forces in in vitro cell culture experiments is fraught with difficulties, including the estimation of the magnitude, type, and duration of the mechanical perturbations, as well as the inability to apply simultaneous differential forces as occur in vivo. For example, the amount of cyclic stretch that results from a SMC force will also be dependent on the compliance of the blood vessel. In addition, EC mechanotransduction is dependent on the developmental stage and vascular bed of the EC investigated. Therefore in vitro studies must be correlated with observations made in vivo in clinically relevant models of human CHD.

**PRESSURE VS FLOW: IN VIVO STUDIES**

**Animal Models**

To understand the impact of increased pressure, flow, or both on the pulmonary vasculature, animal models of CHD provide insight on the progression and mechanisms of PVD and allow for preclinical testing of pharmacologic or other interventions. Low pulmonary blood flow, high pulmonary arterial pressure and resistance, and a dominant right ventricle characterize normal fetal physiology. At birth, dramatic changes in pulmonary blood flow (PBF) patterns occur, most notably a rise in PBF and decline in vascular resistance. Associated with these changes are dramatic changes in gene expression patterns, including cascades that have been implicated in the development of PVD. However, in the setting of CHD these birth-related changes are altered; a delayed increase in PBF after birth is well characterized. Therefore, in order to truly simulate CHD, fetal creation of the defects is essential. To this end, we initially created a model of increased PBF and pressure by placing a large Gore-Tex graft between the ascending aorta and pulmonary artery in late-gestation fetal lambs. This model mimics lesions such as a large VSD. Not only does the physiology of this model mimic infants with common CHD, the biochemical and gene expression alterations described also mimic infants with CHD. To investigate the in vivo effects of flow alone, we have recently developed an ovine model of increased PBF to the right lung following in utero ligation of the left pulmonary artery. Our preliminary data demonstrate the expected physiologic differences in these models (Table 2). Shunt lambs have both increased PBF and pressure, while the right lungs of LPA ligation lambs have increased PBF with a very modest increase in pressure. Importantly, the

<table>
<thead>
<tr>
<th></th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>MAP (mmHg)</th>
<th>HR bpm</th>
<th>PA SBP (mmHg)</th>
<th>PA DBP (mmHg)</th>
<th>MPAP (mmHg)</th>
<th>Δ PAP (mmHg)</th>
<th>RPAQ (L/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>97±12</td>
<td>57±8.6</td>
<td>70±9.4</td>
<td>118±21</td>
<td>20±3.4</td>
<td>8.5±1.6</td>
<td>14±1.8</td>
<td>11.8±0.2</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td><strong>LPA</strong></td>
<td>106±17</td>
<td>58±14</td>
<td>74±15</td>
<td>121±16</td>
<td>27±5.2*</td>
<td>12±3.3*</td>
<td>19±3.6*</td>
<td>14.8±3.2</td>
<td>1.4±0.3*</td>
</tr>
<tr>
<td><strong>Shunt</strong></td>
<td>118±5.7*</td>
<td>36±8.5*</td>
<td>61±8.7</td>
<td>126±17</td>
<td>35±9.2*</td>
<td>18±5.1*</td>
<td>26±6.3*</td>
<td>18±0.4*</td>
<td>2.0±0.2*</td>
</tr>
</tbody>
</table>

P<0.05 vs control. *P<0.05 shunt vs LPA ligation lambs. For control and shunt lambs, right pulmonary artery pulmonary blood flow (RPAQ) was estimated assuming 55% of total PBF to the right lung. SBP=systolic blood pressure; DBP=diastolic blood pressure; MAP=mean arterial pressure; HR=heart rate; PA SBP=pulmonary artery systolic blood pressure; PA DBP=pulmonary artery diastolic blood pressure; MPAP=mean pulmonary arterial pressure; Δ PAP=pulse pulmonary pressure; MPAQ=main pulmonary artery blood flow; RPAQ=right lung pulmonary artery blood flow.
pulmonary pulse pressure is only elevated in shunt lambs. To begin to investigate the effects of pressure + flow vs flow alone on endothelial function in vivo we compared ET-1 and NO production in shunt, LPA ligation, and age-matched control lambs. Interestingly, ET-1 levels are increased in shunt lambs, but not in LPA ligation lambs. Correlative in vitro studies demonstrate that cyclic stretch applied to normal pulmonary artery endothelial cells (PAECs) increases ET-1 levels, while shear stress decreases ET-1 levels. Not surprisingly, eNOS protein expression is increased in both shunt and LPA lungs, which likely represents flow (shear stress) eNOS induction. However, NO metabolite (NOx) levels are increased in LPA lungs, but decreased in shunt lungs (data not shown). These data suggest eNOS uncoupling in shunt lambs, as we have previously described, but maintenance of eNOS coupling in LPA ligation lambs.

We next sought to examine the gene expression profile of PAECs, which are primarily affected by both shear (increased PBF) and cyclic stretch (increased pulmonary pressure.) We first performed RNA sequencing on PAECs derived from control, LPA, and shunt lambs. Principal clustering analysis (Figure 2A) demonstrated excellent differentiation between PAECs derived from each model, as did dendrogram and unsupervised hierarchical clustering heat map analysis (Figure 2B). These data provide visualization for transcriptome-level differences between models. Although important differences exist, the LPA ligation model (increased pulmonary arterial flow only) is the most similar to control, while shunt lambs (increased pulmonary arterial pressure and flow) have more differences in RNA expression, both in terms of significance and fold change.

CONCLUSION

The natural history of pulmonary vascular disease associated with CHD suggests distinct pathophysiological consequences of different hemodynamic insults to the pulmonary vasculature. Classic in vitro studies demonstrate significant differences in the endothelial response to differing types, duration, and magnitude of biomechanical forces. Our preliminary in vivo studies demonstrate substantial differences between the animals with normal physiology, those with increased pulmonary blood flow alone (LPA), and those with increased pulmonary pressure and flow (shunt) both in NO/ET-1 signaling, and in the proximal pulmonary artery endothelial cell transcriptome. Given the significant burden of PVD among patients with CHD particularly in the pediatric population, a fundamental understanding of the differing mechanisms leading to vascular pathology associated with different CHD lesions provides an essential tool in tailoring therapy to these patients. As medicine is increasingly focused on personalized and precision approaches, improved in vitro techniques, and improved animal models of CHD are needed to separate the effects of differential mechanical forces on the pulmonary vasculature. These data may yield important mechanism-specific therapeutic strategies for patients with differing CHD as well as other forms of PVD.

References


