Vascular Remodeling and Reactivity in PPHN

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Neonatal Respiratory Failure in the United States

- 18/1000 live births
- 80,000 neonates/year (2% of all live births)
  - ARDS 40-150,000/year in the US
  - High cost: $4.4 billion
- Overall mortality 11.1%
- 8,500 deaths
  - 1/3-1/2 of all neonatal deaths in the US
Neonatal Respiratory Failure in the United States

- Approximately 2% of all live births will require ventilator support
- ~ 1/3 of ventilated babies are term or near term

Angus et al; AJRCCM 2001;164: 1154.
Normal Fetal Circulation

- Lower Body
- Kidneys
- Lungs
- Brain
- Placenta

Percent Combined Ventricular Output
Normal Fetal Circulation

- Gas Exchange by placenta
- 30-50% of venous blood passes through ductus venosus to bypass blood directly to heart
- Right ventricular output maintained by R⇒L shunts across the foramen ovale and ductus arteriosus
Regulation of Fetal PVR

- Low oxygen tension
- Decreased production of vasodilators
  - Nitric Oxide
  - Prostacyclin
  - Adenosine
- Increased production of vasoconstrictors
  - Endothelin-1
  - Leukotrienes
Fetal Pulmonary Vascular Response to Oxygen
The Transitional Pulmonary Circulation

- PA Pressure
- Blood Flow
- Oxygen Tension

Fetal Life vs. Newborn
Persistent Pulmonary Hypertension in the US

- 1.9/1000 infants (range 0.5-6/1000)
- ~10% of all neonates with respiratory failure
- No genetic factors identified
- High morbidity, mortality
- At risk for long-term sequelae
Pathophysiology of Newborn Pulmonary Hypertension

- Maladaptation: vasoconstriction of a normal vasculature
- The abnormally remodeled vasculature
- The hypoplastic vasculature
- Postnatal remodeling
Antenatal Remodeling Associated With PPHN

Histological Changes Associated With PPHN

PPHN Therapies

- Optimal lung recruitment
  - High frequency ventilation
  - Surfactant
- Cardiovascular support
- Pulmonary Vasodilation:
  - Oxygen
  - Inhaled Nitric Oxide
- ECMO
Cardiopulmonary Interactions in PPHN

- Right-to-left shunting at PDA or FO
- Hypoxia, hypercarbia, acidosis
- Pulmonary vasculature: Structural changes; altered reactivity
- Heart: RV pressure overload, LV dysfunction
- Lung: ↓Lung volume, ↓Compliance, ↑Intrapulmonary shunt
- Heart and Lung: ↑Right-to-left shunting at PDA or FO
- Pulmonary vasculature: ↑PVR, ↓SVR
- Hypoxia, hypercarbia, acidosis

SVR: Systemic Vascular Resistance
PVR: Pulmonary Vascular Resistance
Birth-Related Stimuli: $O_2$, Ventilation, Shear Stress

NO Synthase

Endothelial Cell

NO Synthase

Guanylate Cyclase

GTP $\Rightarrow$ cGMP

PDE5

Vasorelaxation

5$'GMP

Smooth Muscle Cell

PPHN: NO synthase

NO
eNOS Expression is Decreased in Infants that Develop PPHN

Control

PPHN

Effect of iNO on Incidence of Death or ECMO

% Death or Need for ECMO

- NINOS
- CINRG1

- Placebo
- Nitric Oxide
Therapies Prior to ECMO

## Inhaled NO and PPHN Outcome

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>OI</th>
<th>% ECMO Control</th>
<th>% ECMO iNO</th>
<th>% Mortality Control</th>
<th>% Mortality iNO</th>
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<td>NINOS</td>
<td>235</td>
<td>44</td>
<td>55</td>
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<td>48*</td>
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<td>24.7</td>
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<td>Konduri</td>
<td>299</td>
<td>19.2</td>
<td>12</td>
<td>10</td>
<td>9</td>
<td>7</td>
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# Neurodevelopmental Outcomes: Early NO Trial

<table>
<thead>
<tr>
<th></th>
<th>iNO n=121</th>
<th>Control n=113</th>
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</thead>
<tbody>
<tr>
<td>NDI</td>
<td>27.9%</td>
<td>24.6%</td>
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<tr>
<td>MDI&lt;70</td>
<td>25.2%</td>
<td>22.9%</td>
</tr>
<tr>
<td>Hearing Impairment</td>
<td>23%</td>
<td>24%</td>
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</tbody>
</table>

*Konduri et al, J Pediatr 2007*
Mechanisms for Poor NO Response

- Poor Lung Inflation
- Anatomic Lung Disease
- Anatomic Heart Disease
- Right Ventricular Failure
- Left Ventricular Failure
- Alterations in Downstream Signaling
Emerging Insights

Vascular Abnormalities

- NOS Dysfunction
- sGC Dysfunction
- Abnormal cAMP signaling
- Increased phosphodiesterase activity
- Increased rho-kinase activity
- Oxidant stress

New therapies

- NOS Enhancers
- Direct sGC activators
- Prostacyclin analogues, PDE inhibitors
- Phosphodiesterase Inhibitors
- Rho-kinase inhibitors
- Antioxidants
Ductal Ligation Lamb Model

126-128 day gestation pregnant ewe

Lateral thoracotomy and ligation of ductus arteriosus

Induces an acute increase in PBF and development of PPHN

Cesarean delivery 8-13 days after ligation
Ductal Ligation Model of PPHN:

- Severe pulmonary Hypertension
- No parenchymal lung disease
- Hypoxemia unresponsive to O_{2} and ventilation
- Mortality >50% in first 24 hours of life without specific therapy
Remodeled Pulmonary Vasculature in PPHN Lambs
Pulmonary Artery Relaxations in PPHN

Steinhorn et al; Am J Physiol 1994
Birth-Related Stimuli: O₂, Ventilation, Shear Stress

Endothelial Cell

NO Synthase

NO

Endothelial Cell

NO Synthase

PPHN:

↓ NO synthase

sGC

↑ PDE5

Smooth Muscle Cell

Guanylate Cyclase

GTP

cGMP

Vasorelaxation

PDE5

5’GMP
NO Synthase → NO → cGMP → Relaxation

ONOO⁻ → O₂⁻ → SOD → H₂O₂ → Catalase → H₂O

H₂O₂ → Constriction
Vascular Superoxide Production is Increased in PPHN Lambs

Control

Ductal Ligation

Brennan et al; Circ Res 2003
NOS: A Potential Source of Superoxide Production

Konduri et al; Am J Physiol 2003

Graphical representation of the coupled and uncoupled processes involving NOS, L-Arg, NADPH, AMPK, Akt, and Hsp90.
**p67phox Expression is Increased in PPHN Lambs**

Brennan et al; Circ Res 2003
p22 and Nox4 Levels are Increased in PPHN PASMC

Wedgwood et al; PAS 2010
Total SOD Activity in PPHN Lambs

Brennan et al; Circ Res 2003
SOD Isoforms

- SOD1 - CuZn SOD, cytosolic
- SOD2 - MnSOD, mitochondrial
- SOD3 - ecSOD, extracellular
**ecSOD Activity in PPHN Lambs**

**Expression**

- **Fetal Control**
  - [Image]
- **Fetal Ligated**
  - [Image]

**Activity**

- **Control**
  - [Image]
- **PPHN**
  - [Image]

*Wedgwood et al, PAS 2010*
NO Synthase → NO → NO Synthase

ONOO− → O2− → ONOO−

SOD → H2O2 → Catalase

H2O

NADPH Oxidase Mitochondria Xanthine Oxidase

Constriction

Relaxation
rhSOD Treatment of PPHN

![Graph showing a/A Ratio over time for two groups: 100% O2 (n=6) and 100% O2 + NO (n=5). The graph illustrates the trend in a/A Ratio over time, with time in hours on the x-axis and a/A Ratio on the y-axis. Significant differences are indicated by asterisks (*) and double asterisks (**) at specific time points.](image-url)
rhSOD Treatment of PPHN

- 100% O2 (n=6)
- 100% O2 + NO (n=5)
- 100% O2 + SOD (n=4)
rhSOD Treatment of PPHN

- 100% O2 (n=6)
- 100% O2 + NO (n=5)
- 100% O2 + SOD (n=4)
- 100% O2 + Delayed SOD (n=5)

A/A Ratio vs Time (hours)

Lakshminrusimha AJRCCM 2006
Vascular Effects of rhSOD in PPHN Lambs

DHE

100% O2

NO

rhSOD

3NT

100% O2

NO

rhSOD

Lakshminrusimha et al, AJRCCM 2006
rhSOD Restores eNOS Expression

Farrow et al. AJP Lung Cell 2008
PDE5 Inhibition: Rationale

- Enhancement of NO effect
- Protection from NO Toxicity
- Protection from rebound pulmonary hypertension
- Potential for pulmonary vasodilatation
PDE5 Expression During Development

Sanchez et al, Pediatr Res 1998
PDE5 Expression and Activity in PPHN Fetuses

Farrow, et al., Am J Physiol 2010
Hyperoxic Ventilation Increases PDE5 Expression in Normal Lambs

Farrow et al; Circ Res 2008
Oxygen: The Treatment or the Problem?
Exposure to 95% O₂ Increases ROS in FPASMC

Farrow et al; Circ Res 2008
Hyperoxia Increases PDE5 Expression and Activity in FPASMC

Farrow, et. al. Circ Res. 2008
100% Oxygen Blunts Response to NO in PPHN Lambs

Lakshminrusimha et al; Pediatr Res 2009
Increased ROS in PPHN PASMC

Farrow et al, Resp Physiol Neurobiol 2010 in press
Ventilation with 100% $O_2$ in PPHN Lambs

ROS

1DSB  Fetus  100% $O_2$

PPHN

PDE5 Expression

PDE5 Activity

* Farrow et al; Am J Physiol 2010
Birth-Related Stimuli: $O_2$, Ventilation, Shear Stress

Endothelial Cell

NO Synthase → NO

Smooth Muscle Cell

$\text{NO Synthase} \downarrow \text{NO synthase}$

$\downarrow \text{sGC}$

$\uparrow \text{PDE5}$

Guanylate Cyclase

GTP → cGMP

Sildenafil

Vasorelaxation

5'GMP

PPHN:

$\downarrow$ 5'GMP

$\downarrow$ NO

$\uparrow$ PDE5
Oral Sildenafil for PPHN

Baquero et al, Pediatrics, 2006
Intravenous Sildenafil for PPHN

- Five centers enrolled 36 neonates with PPHN or hypoxemic respiratory failure in eight ‘step up’ treatment groups.
- Demographic data for infants:
  - 39±2 weeks gestation
  - 3.44 ± 0.51 kg
  - 34±17 hours of age at enrollment
- Of the 36 infants, 29 were enrolled while already receiving inhaled NO.
Intravenous Sildenafil Improves Oxygenation in PPHN
Response to Sildenafil Infusion without iNO

**Oxygenation Index**

- **Time (Hours):** 0 1 2 4 8 12 24 36 48 60 72
- **Y-axis:** Oxygenation Index

*Note:* The graph shows a decrease in oxygenation index over time with standard error bars. Significant changes are indicated by * and †.
ROS Impair Normal Transition

PPHN 100% O₂

↑↑↑ ROS

↓ eNOS Expression

↑↑↑ PDE5 Activity

Blunted cGMP Vasoconstriction

SOD ± catalase mimetics

Sildenafil
Joseph Priestly, 1774

“…..the new air might not be proper for use in the healthy state of the body........the air which nature has provided us may be as good as we deserve.”
Northwestern University
Stephen Wedgwood
Kathryn Farrow
Paul Schumacker
Nicolas Porta
Greg Waypa
Marta Perez
Lyubov Czech

SUNY Buffalo
Satyan Lakshminrusimha
James A. Russell
Sylvia Gugino

Tufts
Jonathan Davis

Denver
John Kinsella
Stephen Abman