Chronic Lung Disease of Prematurity: Where have all the Vessels gone? Role of Angiogenic Growth Factors, Nitric Oxide and Endothelial Progenitor Cells

Faruqa Ladha, Anu Vadivel, Rajesh Anthuvan, Shumei Zhong, Bernard Thébaud
Children’s Hospital of Eastern Ontario/Division of Neonatology
Ottawa Hospital Research Institute/Regenerative Medicine Program
Sprott Centre for Stem Cell Research
University of Ottawa

Lung Development, Injury and Repair
Increased survival, but no decrease in morbidity for extreme premature infants

<table>
<thead>
<tr>
<th>Gestational age (weeks)</th>
<th>1996-1997</th>
<th>2006-2007</th>
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<tr>
<td>≤ 22</td>
<td>34.7%</td>
<td>46.1%</td>
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Shah, P et al. Canadian Neonatal Network. 2010

BPD: An Arrest in Lung Development

Decreased Alveolarization in BPD: Potential Long Term Consequences

Alvolar Number (x 10⁶)

Normal

BPD
Late Cardiopulmonary Disease in a Young Adult with BPD

Angiogenic growth factors and the making of a blood vessel

HIF
Primary vasculature
Mature, stable vessel
Mature unstable vessel
VEGF
Ang 1
Pericyte
Inhibition of VEGF reduces lung size, impairs alveolar development and decreases lung angiogenesis:

Evidence that angiogenic growth factors promote lung development

**In vivo** intratracheal adenovirus-mediated VEGF gene transfer promotes lung vascular growth
**In vivo** intratracheal adenovirus-mediated HIF gene transfer promotes lung vascular growth

Angiogenic growth factors and the making of a blood vessel

Inhaled NO improves lung structure and function in chronically ventilated preterm lambs

Lin et al., Pediatr Res 2005

Kinsella et al., Schreiber et al., NEJM 2006

NO

Ad.GFP

Ad.HIF

VAdivel et al., Am J Respir Crit Care Med. 2005

+ Ad.GFP

+ Ad.HIF

Bland et al., Am J Respir Crit Care Med, 2005

- Single centre RCT
- 207 newborns < 34 wks, <2000g, requiring MV
- iNO 10ppm for 1d then 5ppm for 6 d or placebo gas
- 1° outcome death or BPD at 36 weeks
- 2° outcome IVH, PVL and PDA
- Main findings:
  - iNO ↑ survival without BPD (p=0.03/R 0.76 [95%CI 0.60-0.97])
  - especially among infants with less severe lung disease (OI< 7) (p=0.02/R 0.53 [95%CI 0.35-0.81])
  - 47% decrease in severe intracranial hemorrhage and PVL


- 138 children (82 % of survivors)
- abnormal neurodevelopmental outcomes defined as either:
  - disability (CP, bilateral blindness, or bilateral hearing loss) or
  - delay (no disability, but one score < 70 on the Bayley Scales of Infant Development II)
- iNO 24 % abnormal vs 46 % in the placebo group (RR 0.53; 95 percent confidence interval, 0.33 to 0.87; P=0.01)
- Effect persisted after adjustment for BW, gender, presence or absence of BPD and severe IVH or PVL
Medical and Functional Outcomes at Early School-Age:

- 135 of 167 survivors (81%) at ≈ 6 years.
- Medical outcomes: somatic growth, hospitalizations and ongoing morbidities
- Functional outcomes: School readiness levels using standardized neurodevelopmental assessments of basic concepts (Bracken School Readiness Component), perceptual skills (Visual-Motor Integration), receptive vocabulary (Peabody Picture Vocabulary Test-3rd Ed.), daily living functional skills (WeeFIM), cerebral palsy, hearing impairment, vision impairment, and autism

- Compared to placebo (n=65), iNO children (n=70) had no difference in:
  - Weight, height, head circumference; hospitalizations.
- Compared to placebo, iNO children were:
  - more likely to have none/mild/single chronic ongoing morbidity
  - less likely to have multiple chronic ongoing morbidities or technology-dependence
- Functional outcomes were similar between groups


- Multicentre RCT (16 centres)
- 793 newborns < 34 wks, <1250g requiring MV <48H
- iNO 5ppm or placebo gas for 21 d or until extubation*

1° outcome composite of death or CLD at 36 weeks
2° outcome IVH, PVL or ventriculomegaly
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- Overall iNO:
  - ↑ survival without BPD (p=0.04)
  - ↓ LOS (p=0.004), ↓ length on O₂ (p=0.006)
  - ↓ lung disease severity if enrolled from 7-14 d vs 15-21d

Effects of iNO on survival without BPD for infants enrolled between 7 and 21 days of age


Modified by Kinsella et al. J Pediatr 2007
### EUNO. et al. Lancet 2010

- Multicentre RCT (36 EU centres)
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### Kinsella et al, J Pediatr 2014

- To assess the efficacy and safety of early, noninvasive inhaled nitric oxide (iNO) therapy in premature newborns who do not require mechanical ventilation
- Multicentre RCT, 124 newborns who required noninvasive supplemental oxygen within the first 72 hours after birth
- Stratified by birth weight (500-749, 750-999, 1000-1250 g) prior to randomization to iNO (10 ppm) or placebo gas (controls) until 30 weeks postmenstrual age
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### Summary of iNO Trials

- **Early rescue therapy for severe respiratory failure:**
  - No benefit
  - Potential increased risk of severe ICH
  - Selective indications? PPROM ± oligohydramnios

- **Prevention of BPD in infants with mild respiratory distress:**
  - Safe
  - Effective in a subset of preterm infants>1000g
  - Neuroprotective
  - Economical considerations (high cost barrier to its use?)
L-Citrulline preserves alveolar development in O₂-induced BPD in newborn rats

Angiogenic growth factors and the making of a blood vessel
**Endothelial Progenitor Cells (EPCs):**

ECFCs fulfill the stringent criteria of a true EPC

ECFC hierarchy model based on proliferative and clonogenic potential of discrete populations of progenitor cells

ECFCs exist in the Human Fetal Lung

Cobblestone-looking ECFC Colony

LDL-uptake and Ulex-lectin binding

Cell Surface Markers

Endothelial network formation

CD31
CD146
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CD14 CD45

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Network Formation

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**Therapeutic Potential of UCB-ECFC in Experimental O₂-induced Neonatal Lung Injury**

- Endpoints:
  - Lung Compliance (Raw/cm H₂O)
  - Lung Histology
  - Pulmonary artery acceleration time
  - Right ventricular hypertrophy

**Human cord blood-derived ECFCs Restore Arrested Alveolar and Lung Vascular Growth in O₂-induced Experimental BPD**

**Human cord blood-derived ECFCs Attenuate Pulmonary Hypertension in O₂-induced Experimental BPD**
Resident Lung ECFC Function is Restored by Exogenous Human cord blood-derived ECFCs

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Low Lung Engraftment of Cord Blood-derived ECFCs
Evidence that ECFCs act via a Paracrine Effect:
Therapeutic Potential of cell-free Conditioned media

- In vitro
  - 1. AEC wound healing
  - 2. EC capillary-like network

Endpoints
- Lung Compliance (Blackeart™)
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Angiogenesis promotes lung growth and repair
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1. Angiogenesis inhibition

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Lung Development, Injury and Repair

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William H. Northup, Jr., M.D.1, Robert C. Koblin, M.D.2, and Steve V. Porter, M.D.1

PULPE ALTA, CALIFORNIA
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Shah, P et al. Canadian Neonatal Network. 2010

Northway et al, NEJM 1967 Survivors with BPD: 34 GA, 2234g Non-survivors: 31 GA, 1660g

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The "new BPD" Jobe A. Pediatr Res 1999

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Slide courtesy Steven Abman

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Effects of iNO on survival without BPD for infants enrolled between 7 and 21 days of age


- Entire Study Population (N = 582 patients)
  - p=0.042

EUNO. et al. Lancet 2010

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Alphonse RS et al. Circulation 2014

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Endpoints
- Lung Compliance (R Rihanna2)
- Lung Histology
- Pulmonary artery acceleration time
- Right ventricular hypertrophy

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Alveolar Growth
- RA
- RA+ECFC
- O₂
- O₂+ECFC

Vascular Growth
- RA
- RA+ECFC
- O₂
- O₂+ECFC

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Right Ventricular Hypertrophy
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Study periods

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Alveolar Number (x 10^6)

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A) Mean Linear Intercept

B) Septal counts

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HIF

VEGF

NO

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Network Formation

Clonogenic Potential

0
5
10
15
20
25
Room Air

40% O₂

Nb Intersections

*P<0.05

ECFC Function is impaired in O₂-induced BPD

Experimental O₂-induced “BPD” in Newborn Rats

O₂

95% Oxygen

Birth (P0)

Room Air

60% O₂ -induced BPD model

Alveolar stage (P14-21)

P<0.05

Alveolar structure

Lung vascular structure

H&E staining

Scanning electron

EEC/ECF micrograph

Bowel architecture

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