Endothelin-1-Rho-kinase Interactions Impair Angiogenesis in Fetal Pulmonary Artery Endothelial Cells after Chronic Intrauterine Pulmonary Hypertension

Jason Gien MD, Gregory Seedorf BS, Neil Markham BS, Nancy Tseng MS, Steven Abman MD, Pediatric Heart Lung Center, UCHSC and The Children’s Hospital, Denver, Colorado

INTRODUCTION
Persistent Pulmonary Hypertension of the Newborn (PPHN)

- PPHN is a clinical syndrome characterized by:
  - elevated pulmonary vascular resistance (PVR)
  - reduced pulmonary blood flow
  - elevated PVR may be due to:
    - increased vascular tone
    - hyperplastic remodeling
    - impaired vascular growth, or angiogenesis

Rho-kinase and Endothelin-1 in Pulmonary Hypertension

- Rho-kinase activation maintains PVR in the normal fetus and contributes to high PVR in PPHN.
- PPHN caused by in utero ductal closure is characterized by increased fetal ET-1 levels.
- Endothelial cell Rho-kinase activity is increased in PPHN caused by ductal closure and impairs angiogenesis in this model.

HYPOTHESIS
Increased production of ET-1 by PAECs from experimental PPHN increases PAEC Rho-kinase activity ad impairs angiogenesis in vitro

STUDY QUESTIONS
1) Is ET-1 production and ET receptor B expression altered in PAECs harvested from PPHN Lams?
2) What is the effect of ET-1 on rho-kinase activity, NO production and phosphorylated eNOS protein expression in normal and PPHN PAECs?
3) What is the effect of ET-1 on tube formation in vitro and is this effect altered in the presence of rho-kinase inhibitor?

METHODS
Isolation of Pulmonary Artery Endothelial Cells

- Pulmonary artery endothelial cells are harvested from late gestation (135-145 days) fetal lambs using a modified method.
- 24 hour exposure — ET-1 (100nM)

Western Blot Analysis — P-MYPT-1, eNOS

- Levels obtained from normal and PPHN endothelial cells
- 24 hour exposure — ET-1 (100nM)

Tube Formation Assay

- PAECs when mixed in collagen will form tubes within the collagen matrix over a 10-14 day period.
- 5 x 10^5 endothelial cells are mixed in collagen.
- The number of branch points and tube length are then counted.

RESULTS
- ET-1 Production is increased in PAECs from PPHN Lams
- ET-1 decreases P-eNOS protein expression and NO production by Normal and PPHN PAECs
- Endothelin-1 decreases Tube Formation in vitro

Conclusions
- PPHN directly alters PAEC phenotype.
- ET-1 production is increased in PPHN PAECs and activates rho-kinase, decreases P-eNOS protein and NO production and impairs angiogenesis.
- ET-1-rho-kinase interactions impair angiogenesis after chronic intrauterine pulmonary hypertension.

SPECULATION
- Therapies aimed at decreasing ET-1 production in PPHN will prevent rho-kinase activation and improve angiogenesis in PPHN.