Prostacyclin Analog Treprostinil Enhances Neonatal Rat Lung Endothelial Cell Growth And Angiogenesis *In Vitro*. KG Thayapran (MD, SOM), G Seedorf, SH Abman. *Pediatric Heart Lung Center, Department of Pediatrics, University of Colorado School of Medicine, Aurora CO*.

**Purpose of Study**

Bronchopulmonary dysplasia (BPD) is the chronic lung disease that often follows preterm birth. Characterized by abnormal lung structure due to impaired alveolar and vascular growth, BPD is strongly associated with mechanisms such as postnatal hyperoxia and the risk for pulmonary hypertension (PH). Previously, we found that treprostinil (TRE), a synthetic prostacyclin analog, preserved lung structure and function, improved vascular growth, and prevented right ventricular hypertrophy in a hyperoxia-induced neonatal rat model of BPD. To determine whether the effect of TRE on neonatal lung development is partly due to the stimulation of angiogenesis, we studied the effect of TRE on rat lung endothelial cell (LEC) growth and tube formation *in vitro.*

**Methods Used**

LECs were isolated from 2-week old rats and grown in 10% FBS. To assess cell proliferation, LECs were plated in 2.5% FBS (5000 cells/well), grown in normoxia with daily media changes, and counted after 3 days. To assess angiogenesis, LECs were plated in 1% FBS (10,000 cells/well) on collagen and fixed in 4% PFA after 18-24hrs in normoxia. Cells were imaged at 10x and tube formation was assessed by counting branch points per high powered field. For both assays, the following treatments were studied: untreated FBS (control), TRE (1uM), Axitinib (AX, selective VEGF receptor inhibitor; 10nM), and TRE+AX.

**Summary of Results**

TRE increased LEC growth and tube formation by 109% and 51%, respectively ($p<0.01$ and $p<0.05$). AX alone did not decrease LEC growth, and when TRE was administered with AX, the effect of TRE was not attenuated. However, AX alone decreased tube formation by 38% ($p<0.01$) but TRE administration with AX restored tube formation to control values.

**Conclusions**

TRE enhances LEC growth and angiogenesis *in vitro,* supporting our previous findings that TRE improves lung alveolar and vascular growth *in vivo.* Further, we found that VEGF receptor blockade reduces tube formation but not cell growth*,* but this effect can be reversed by TRE. We speculate that these findings suggest interactions between the VEGF and prostacyclin pathways that can be targeted to develop novel therapies to prevent BPD and BPD-associated PH.