Maternal Vitamin D Deficiency Alters Pulmonary Endothelial Cell Growth and mRNA Expression in Newborn Rats

Gonzalez T1, Bye E1, Seedorf GJ1, Fleet JC2, Abman SH1, Mandell EW1

1Pediatric Heart Lung Center, Department of Pediatrics, University of Colorado, Denver Anschutz Medical Center, Aurora, CO, USA;
2Department of Nutrition Science, Purdue University Center for Cancer Research, West Lafayette, IN

Background

- Vitamin D deficiency (VDD) during pregnancy is increasingly recognized and associated with several maternal and fetal morbidities, such as abnormal airways development, airway hyperreactivity and smaller tracheal diameter. (Yurt M, 2014, Saadon A 2017)
- Antenatal vitamin D (VD) treatment improves distal lung structure and prevents pulmonary hypertension in an experimental model of Bronchopulmonary Dysplasia (BPD). (Mandell EW, 2014)
- Vitamin D treatment also enhances:
  - Fetal pulmonary endothelial cell growth and angiogenesis. (Mandell EW, 2014)
- Vitamin D upregulates pro-angiogenic stimuli in the developing lung, including vascular endothelial growth factor (VEGF) expression. (Mandell EW, 2014)
- However, mechanisms through which maternal VDD impairs distal lung growth and development remain unknown.

Hypothesis

Maternal Vitamin D Deficiency impairs growth and mRNA expression in pulmonary endothelial cells at birth and these changes may persist during infancy.

Study Questions

Does maternal vitamin D deficiency:
1. Decrease growth in pulmonary endothelial cells (PEC) collected at D0 and D14?
2. Alter responsiveness to pro-angiogenic stimuli (VEGF, 1,25-OH VD and 25-OH VD)?
3. Alter basal expression of VDR, KDR, VEGF, and eNOS in PEC at D0 and D14?

Methods

Study Design: Model of Vitamin D Deficiency

Study Measurements
- Isolation of pulmonary endothelial cells (PEC)
  - Harvested from distal lungs of D0 and D14 rat pups
- Magnetic bead expansion for CD31+ cells
- Characterized by cobblestone morphology and PEC markers
- Cells counted at day 3
- Treatments:
  - VEGF (100 ng/ml)
  - 1,25-OH Vitamin D (500 nM)
  - 25-OH Vitamin D (250 nM)
- qPCR: Isolated total RNA and assessed VDR, KDR, VEGF, and eNOS expression in PEC and at D0 and D14

Results

Growth Assay: PEC Responsiveness

Maternal VDD Decreases PEC Responsiveness to VEGF at D0 and D14

Maternal VDD Decreases PEC Responsiveness to 1,25-OH-D at D0 and D14

Maternal VDD Decreases PEC Responsiveness to 25-OH-D at D0 and D14

Summary

- VDD PEC have decreased growth and responsiveness to angiogenic stimulation with VEGF and vitamin D (25-OH-D and 1,25-OH-D) at D0 and D14.
- VDD PEC have no change in VDR RNA expression compared to CTL PEC at D0.
- VDD PEC have increased RNA expression of VDR at D14.
- VDD PEC have decreased KDR and eNOS RNA expression and increased VEGF RNA expression compared to CTL PEC at D0; no changes at D14

Conclusion

Maternal vitamin D deficiency impairs pulmonary endothelial cell growth and alters mRNA expression of VDR, KDR, VEGF and eNOS.

Speculation

Vitamin D is essential to endothelial cell health and antenatal vitamin D deficiency increases the risk for the development of BPD in preterm infants.