Antenatal Mesenchymal Stromal Cell Extracellular Vesicle Treatment Preserves Lung Development in a Model of Bronchopulmonary Dysplasia due to Chorioamnionitis

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Pediatric Heart Lung Center

Background

Bronchopulmonary Dysplasia (BPD):

- BPD is the chronic lung disease of prematurity characterized by early disruption of lung growth and contributes to late morbidity and mortality.
- Although the etiology of BPD is multifactorial, strong evidence has shown that antenatal factors, such as chorioamnionitis (CA), are associated with an increased risk for BPD.
- Antenatal endotoxin (ETX) exposure as an experimental model of CA causes sustained disruption of lung alveolar and vascular growth hallmark findings in BPD.

Mesenchymal Stromal Cell (MSC) Extracellular Vesicles (MEx):

- MEx are secreted membrane vesicles from MSC that modulate many cellular functions, including growth, differentiation and function in health and disease.
- Experimentally, MEx has shown promising effects in preventing or restoring lung function in models of lung disease.
- Postnatal treatment with MEx can improve lung structure in experimental BPD due to postnatal hyperoxia, however, the potential efficacy of MEx for the prevention of BPD due to ***antenatal*** stress is unknown.

Hypothesis

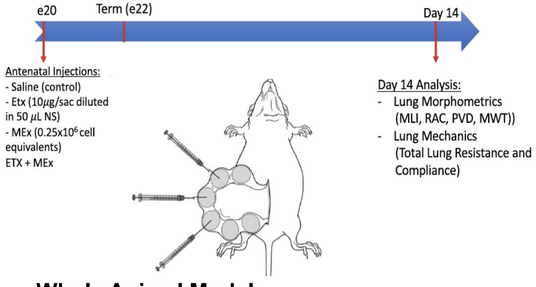
Antenatal MEx treatment will prevent the development of BPD in an experimental rat model of CA.

Study Questions

- Will intra-amniotic injection of MEx:
  - Preserve vessel and alveolar growth and improve lung structure in infant rats exposed to antenatal ETX?
  - Improve lung mechanics in infant rats exposed to ETX?

Methods

- All animal procedures and protocols approved by the Animal Care and Use Committee at the University of Colorado Heath Sciences Center.
- Timed pregnant Sprague-Dawley rats were used for this study.



Whole Animal Model:

Lung Morphometric Analysis

- Lungs were inflated at 20cm H2O for an hour and fixed in 4% paraformaldehyde
- H&E staining used to determine distal lung structure with Mean Linear Intercepts (MLI) and Radial Alveolar Counts (RAC)
- vWF immunostaining used to identify pulmonary vessels for determination of vessel density (VD) and medial wall thickness (MWT); Right ventricular hypertrophy (RVH) calculated

Lung Mechanics Studies

- Flexivent® single compartment analysis used to measure resistance and compliance

***In Vitro* Model:**

Fetal Lung Explants

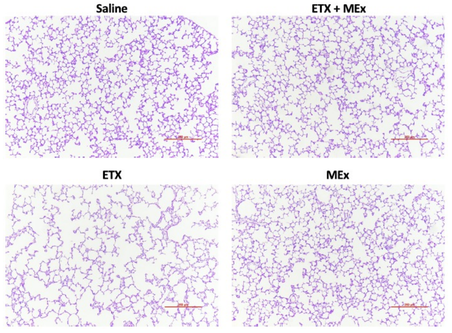
- Lungs harvested at E15 gestation; distal branching was assessed day 0-3

RT PCR

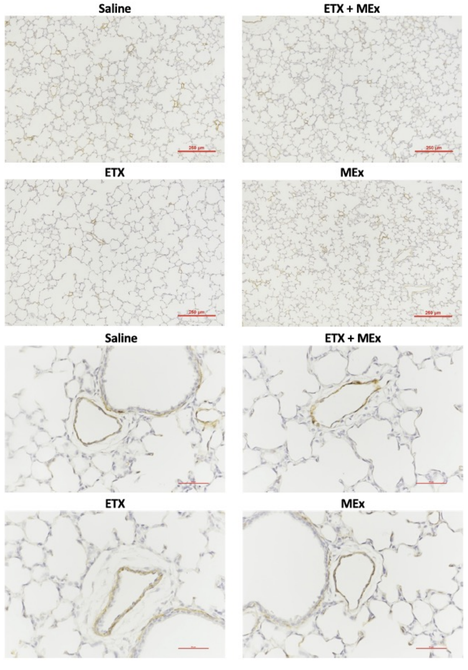
- Total RNA harvested from day 3 lung explants; RT PCR performed using TaqMan Fast Advanced Master Mix (Invitrogen) for GAPDH, SPC, and VEGF

Results

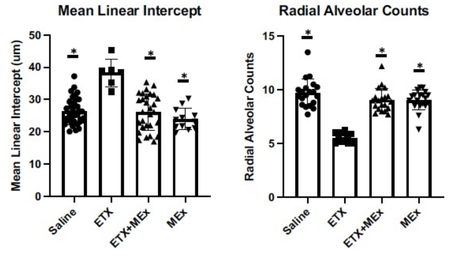
Antenatal MEx Treatment Preserves Lung Alveolar Growth in BPD

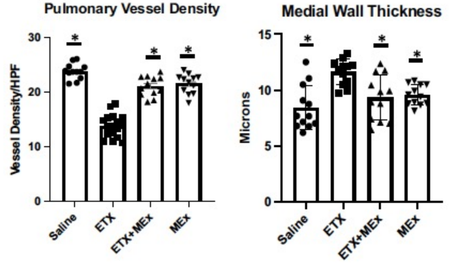


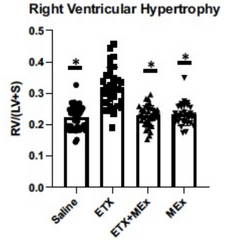
Antenatal MEx Treatment Preserves Lung Vascular Growth in BPD



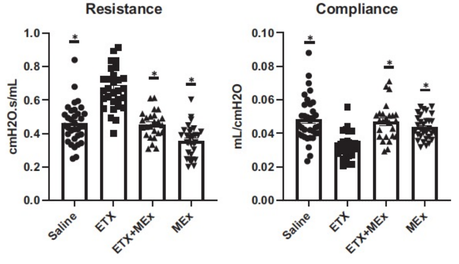
Results





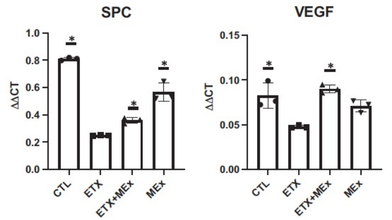


Antenatal MEx Treatment Preserves Lung Mechanics



Results

Antenatal MEx Treatment Increases SPC and VEGF Gene Expression



Summary

- ***Intra-amniotic ETX*** impairs alveolar and vascular growth and mechanics in infant rats.
- ***Antenatal MEx*** injections preserve lung alveolar and vascular growth and restores lung mechanics after ETX-exposure.
- ***Antenatal MEx*** significantly increases SPC and VEGF gene expression in comparison to ETX-exposed samples.

Conclusion

Intra-amniotic MEx preserves lung alveolar and vascular structure and improves lung mechanics in infant rats with experimental BPD induced by antenatal ETX.

Speculation

Early antenatal MEx treatment may prevent the development of BPD in premature infants, especially in the clinical setting of antenatal inflammation.

Disclosures: Exosomes used in these studies are provided in collaboration with the Kourembanas lab at the Department of Neonatology at Boston Children's Hospital, Harvard Medical School, in conjunction with United Therapeutics.