CHRD 2022: Abstract & Poster Submission Form

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Presenter Status

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◉ Non-Trainee

Research Category

◉ Basic Science

○ Clinical

○ Community Health / Policy

Role in the project

☑ Design

☑ Perform Experiments

☑ Analyze Data

☑ Write Abstract

☐

Title

Cellular uptake of breast milk-derived extracellular vesicles is higher in mothers with asthma in a transwell model of the gastrointestinal barrier

Background

Breastfeeding may be protective against asthma development, and components of breast milk (BM) likely play a critical role in determining this effect. An understudied component of BM is extracellular vesicles (EVs), key mediators of cellular communication that can modify recipient cell function. The role of BM-EVs in asthma is unexplored. Our pilot study illustrated anti-inflammatory effects of BM-EVs from mothers with asthma on airway smooth muscle cells.

Objective

To determine whether the effect of BM-EVs is contingent on cellular uptake, we established an in vitro transwell co-culture model to elucidate if BM-EVs: 1) pass intestinal epithelial cell (Caco-2) barrier, 2) are taken up by macrophage cells (THP-1), and 3) if uptake is dependent on maternal asthma status.

Methods

We ensured integrity and uniformity of Caco-2 layer by transepithelial electrical resistance (TEER), fluorescent FITC-dextran quantification and immunofluorescent staining of zona occludens-1 (ZO-1). BM (100 µl) from 3-4mths post-partum mothers with/without asthma (N=3, CHILD study) was used to isolate BM-EVs by size exclusion chromatography, and labeled using fluorescent dye PKH67. Labeled BM-EVs were added to apical layer with Caco-2 cells for 24hrs, and uptake measured in THP-1 cells seeded on the basal side using epifluorescent microscopy.

Results

Differentiation of Caco-2 cells was monitored daily by TEER and reached average resistance 775.62 Ω·cm2 on day seven. Subsequently, FITC-dextran and ZO-1 staining was used to confirm an intact Caco-2 layer. Apical BM-EV treatment did not change TEER ($p>0.05$). Uptake of BM-EVs by basal THP-1 cells was 4.97-fold lower for asthma-BM-EVs vs. non-asthma control-EVs ($p=0.0047$, N=3); this, despite a 7-fold higher asthma-BM-EV concentration ($p<0.05$, N=4-5).

Conclusion

Caco-2/THP-1 transwell system is a promising model to investigate BM-EV uptake and function. Uptake of asthma-BM-EVs is lower across the gastrointestinal barrier. The mechanisms underlying this effect and functional relevance in offspring asthma development remains to be determined.

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