CHRD 2021: Abstract & Poster Submission Form

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Research Category:

- ◉ Basic Science
- ○ Clinical
- ○ Community Health / Policy

What was your role in the project?

- ☑ Design
- ☑ Perform Experiments
- ☐ Analyze Data
- ☑ Write Abstract
- ☐

Presenter Status:

- ◉ Undergraduate Students
- ○ Masters Student
- ○ PhD Student
- ○ Post-Doctoral Fellows
- ○ Residents
- ○ Non-Trainee

Title

Mechanisms for Asthma Pathobiology: The Effects of Oxidized Phospholipids on Mitochondrial Function in Human Airway Smooth Muscle Cells

Background

Asthma is a chronic lung disease characterized by excessive airway narrowing that affects 12% of Canadian children. Oxidative stress, a feature of asthma, causes the peroxidation of phosphatidylcholine a major phospholipid in lung cells and extracellular fluids. Oxidized phosphatidylcholines (OxPCs) are pro-inflammatory and accumulate in the lungs of mice and humans after inhaled allergen challenge. Previous work from our group suggests that OxPC's induce generation of reactive oxygen species and mitochondrial dysfunction in human epithelial cells.

Objective

Here, we test the hypothesis that OxPCs exposure affects mitochondrial function, and membrane permeability in cultured human airway smooth muscle cells (HASMC).

Methods

MTT assay was used as a measure of HASMC viability after 24 and 48 hr exposure to OxPCs, specifically, OxPAPC (ie. oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine), and PSPC (1- palmitoyl-2-stearoyl-sn-glycero-3-phosphocholine), a fully saturated, non-oxidized OxPC. We also used staining with TMRM to assess OxPCs effects on mitochondrial membrane permeability, and MitoSox to measure mitochondrial superoxide production. Fluorescent signal intensity was measured using fluorescent microscopy and flow cytometry. For all experiments we used three different HASMC cell lines.

Results

Neither OxPAPC nor PSPC (10-160 ug/mL) significantly affected HASMC viability after 24 and 48hrs. Conversely, OxPAPC (80 and 160 ug/mL) appeared to induce mitochondrial hyperpolarization, evident from an increase in the median fluorescent intensity for TMRM (measured by flow cytometry), by more than 100% and 150%, respectively, compared to untreated cells (and an increase from PSPC-exposed cells). Similarly, the median fluorescent intensity of MitoSox in HASMC exposed to OxPAPC (80 and 160 ug/mL) increased approximately 50%, and 75%, respectively, compared to MitoSox stained control cells, and PSPC-exposed cells. This indicates mitochondrial generation of reactive oxygen species.

Conclusion

These findings demonstrate that OxPCs affect mitochondrial membrane permeability, as well as mitochondrial superoxide production. This implicates a role for asthma-associated OxPCs in affecting mitochondrial function in HASMC.

Authors

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