CHRD 2022: Abstract & Poster Submission Form

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Presenter Status

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

Research Category

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

Role in the project

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

Title

Use of a Novel Live Cell Oxygen Consumption Rate Monitor to Assess Effects of Oxidized Phosphatidylcholine on Mitochondrial Function in Lung Cells

Background

Asthma is the most common chronic disease and primary reason for childhood emergency room visits in Canada. Mitochondrial dysfunction and oxidative stress are linked with asthma pathophysiology. Oxidative stress damages airway phospholipids generating oxidized phosphatidylcholine (OxPC), which can induce mitochondrial dysfunction in airway cells.

Objective

This study aims to characterize baseline metabolism of human airway smooth muscle (HASM) from asthmatic donors and a human epithelial cell line using oxygen consumption rate (OCR) and OxPC-exposure responses measured with a novel OCR reader.

Methods

Confluent primary and matched human telomerase reverse transcriptase (hTERT)-HASM, two healthy, one mild-, and two moderate-asthmatic donors and Calu-3 human lung epithelial carcinoma cells were seeded into 96-well plates (20,000 cells/100µL Dulbecco's Modified Eagle Medium(DMEM)/10% fetal bovine serum(FBS)). Following overnight incubation, OCR was recorded for 24-hours using a RESIPHER OCR monitor. OCR was further recorded for Calu-3 cells (DMEM/0.5% FBS) for 6-hours, then cells were treated for 24-hours with OxPC (10, 20, 40, and 80µg/mL) or a non-oxidizable phosphocholine(control). Triplicate experiments were completed. OCR values were analyzed by one-way ANOVA with Tukey correction for multiple comparisons.

Results

Using five matching donor cell lines of asthmatic HASM, OCR values were not significantly different for primary versus hTERT-HASM, nor healthy versus asthmatic-HASM ($\beta=0.95$) after 6-hours of growth, however an initial period of increased hTERT-HASM OCR was evident at 3-hours ($p<0.001$). Calu-3 cells showed dose-dependent decreases in OCR in response to OxPC (20µg/mL:$p<0.05$, 80µg/mL:$p<0.001$) compared to cells in OxPC-deficient medium. Phosphocholine treatment had no effect on OCR.

Conclusion

The RESIPHER live cell OCR monitor is a sensitive and cost-effective tool to assess mitochondrial biology in cultured cells. OCR under growth conditions was similar for primary and hTERT-HASM cells from healthy and asthmatic donors. OCR is decreased by OxPC exposure in human airway epithelial cells, confirming an effect of OxPCs on mitochondrial metabolic function.

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Authors

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