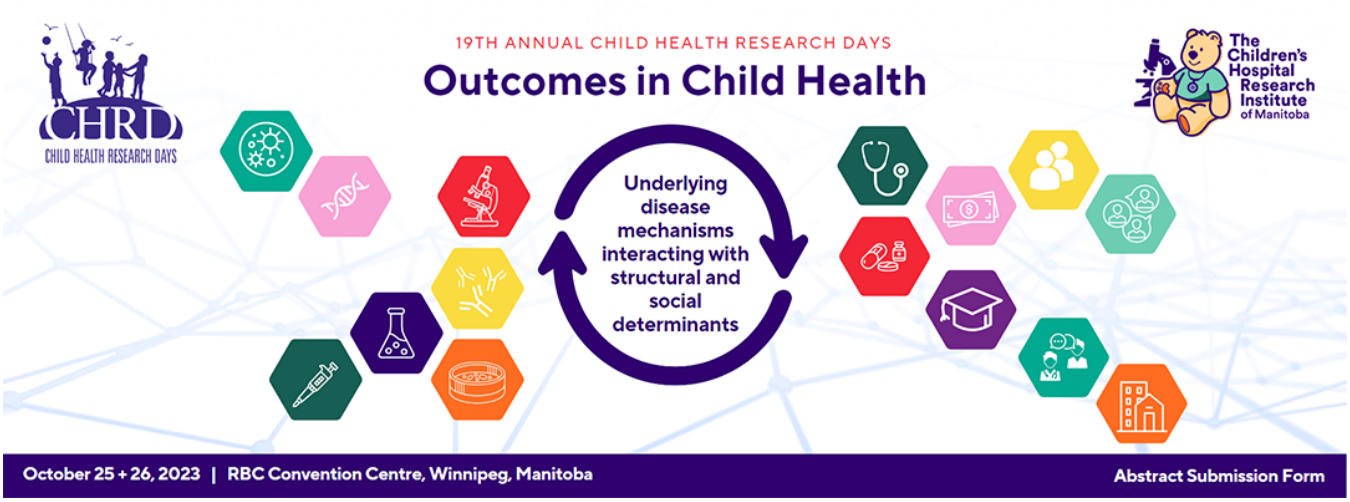

CHRD 2023: Abstract Submission Form

Submitter Name
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Presenter Name
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Presenter Status
Undergraduate Students

Research Category
Basic Science

Role in the project
Design
Perform Experiments
Analyze Data
Write Abstract

Title
Deciphering the Oxidized Phospholipid-Protein Kinase C Signalling Axis in Human Airway Smooth Muscle

Background
According to an Asthma Canada report, approximately 317 Canadians, including children, are diagnosed with asthma daily, and a significant number of them are refractory to current therapies. The mechanism of refractory asthma remains unclear. We previously showed that oxidized phosphatidylcholines (OxPC), a mediator of oxidative stress, exacerbates asthma pathobiology (i.e. increased inflammation, bronchodilator insensitivity) via pathways that involve Protein Kinase C (PKC).

Objective
The present study aims to develop protocols to test whether OxPC exposure is sufficient to activate PKC.

Methods
Cultured Human Airway Smooth Muscle (HASM) cells (n= 5 donors) were treated with OxPAPC (the most common variants of OxPC in the lungs) (80 µg/mL) for 1 to 24 hours. We used untreated cells as a negative control and cells treated with the PKC activator 12-O-Tetradecanoylphorbol-13-acetate (TPA) (0.2uM, 1 hour) as a positive control. After treatment, cell lysates were collected, proteins were separated by 8% SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose by electroblotting, and PKC phosphorylation was detected using polyclonal anti-pSer660-PKC. Using β-actin as a loading control, pPKC bands were quantified by densitometry using AlphaEase FC software and calculated as % p-PKC increased from baseline.

Results
OxPAPC (80 µg/mL) exposure for 1 hour in HASM cells significantly induced PKC phosphorylation (203%±187 increase from baseline), which was comparable to induction with the PKC activator, TPA (256%±216) (Figure 1). Interestingly, OxPAPC (80 µg/mL) treatment for longer periods showed that OxPAPC exposure leads to a sustained increase in PKC phosphorylation (i.e. 222%±169 at 3 hours, 233%±195 at 6 hours, 239%±261 at 24 hours) (Figure 1).

Conclusion
Oxidized phosphatidylcholine causes sustained PKC phosphorylation in HASM cells. This finding confirms the existence of an oxidized phospholipid-protein kinase C signalling axis, furthering our understanding of asthma pathobiology involving mediators of oxidative stress.

Table/Figure File
Figure 1 - NV CHRD Abstract.pdf

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Figure 1: anti-pSer660-PKC % increase from baseline