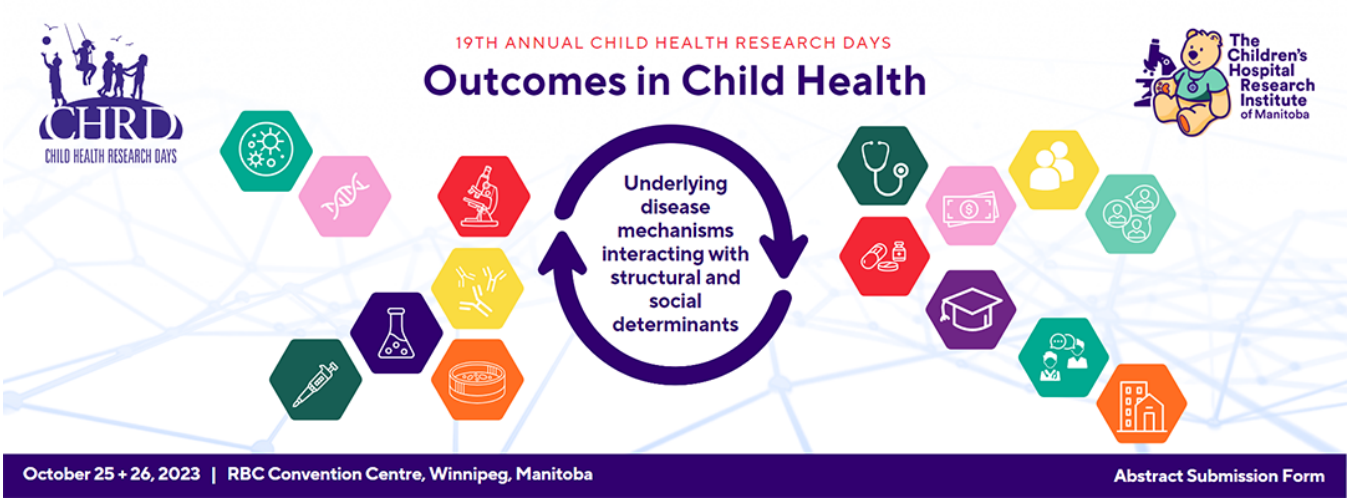

CHRD 2023: Abstract Submission Form

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Presenter Status
PhD Student

Research Category
Basic Science

Role in the project
Design
Perform Experiments
Analyze Data
Write Abstract

Title
Emerging Role of Oxidative Stress Mediators in Reducing the Effectiveness of Asthma Therapy

Background
Oxidative stress modifies phospholipids in the lung, and we reported that inhaled allergen challenge of human participants generates oxidized phosphatidylcholines (OxPC) in lung in concert with airway hyperreactivity. OxPC also induce inflammation, intracellular Ca2+ flux and contraction of human airway smooth muscle (HASM) cells.

Objective
As environmental triggers of oxidative stress associate with therapy failure in asthmatics including children, we tested whether OxPC impairs β2 adrenergic receptor (β2AR) induced bronchodilator responses.

Methods
Murine tracheal rings (n=6, BALB/c) were pre-incubated with OxPC (80 μg/mL, 24 hrs, 37ºC), and the dose-response isometric force relaxation curve for the β2AR agonist, isoproterenol (Iso) was measured. Cultured HASM cells (5 donors) were treated with OxPC (5-80 μg/mL), then Iso (1 nM)-induced cAMP signaling was assayed by VASP-shift and cADDis cAMP live cell kinetics. To identify pathways for OxPC effects, cells or rings were pre-treated with inhibitors for Protein Kinase C (PKC)(GF-109203x) or COX2 (indomethacin). HA-tagged β2AR expressed HEK-293 cells were treated with OxPC and changes in surface β2AR abundance was measured with HRP-conjugated anti-HA IgG. Data analysis included nonlinear-curve-fit or one-way-ANOVA.

Results
In tracheal rings, OxPC significantly increased EC50 of the Iso relaxation dose-response curve (17.8 nM), compared to control (3.3 nM) (Figure 1A). OxPC dose-dependently reduced β2AR-mediated cAMP-signalling in HASM cells: (1) VASP-shift assay - Iso induced 58.5±5.80% p-VASP, but OxPC pre-treatment inhibited p-VASP by 70% (17.0±1.87%), and (2) cADDis cAMP-kinetic assays - OxPC reduced Iso-induced cAMP by ~50%. Surface β2AR abundance in HEK cells was unchanged by OxPC treatment. PKC inhibition prevented the suppressive effects of OxPC on Iso-induced cAMP signalling of HASM cells and relaxation of tracheal rings (Figure 1B).

Conclusion
OxPC impairs □2AR-agonist induced bronchodilation in association with PKC activity and suppression of cAMP signaling. This suggests that OxPC-induced PKC activity leads to β2AR desensitization, which could underpin bronchodilator insensitivity in asthma therapy.

Table/Figure File
Figure 1.pdf

Authors

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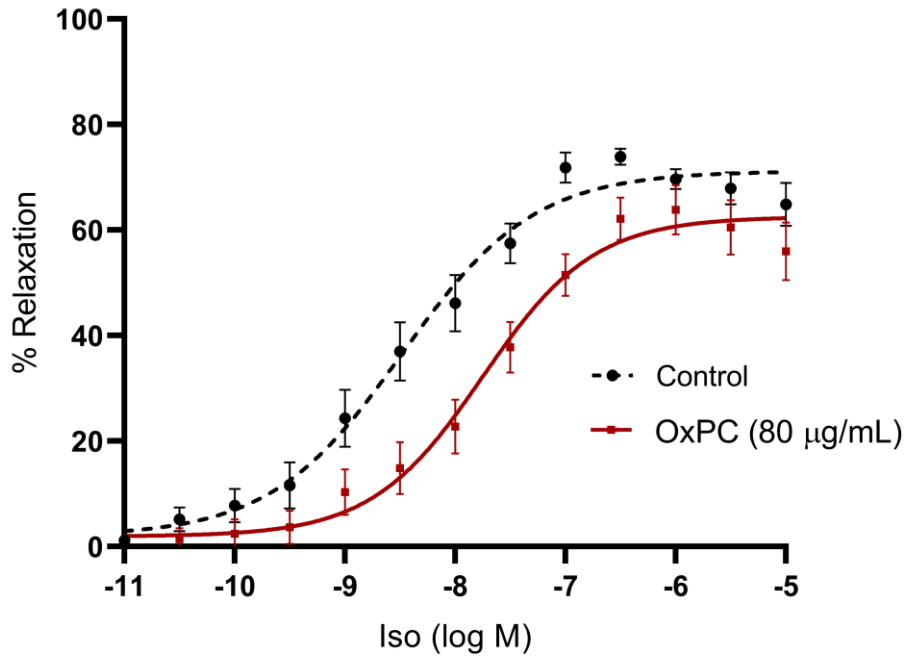
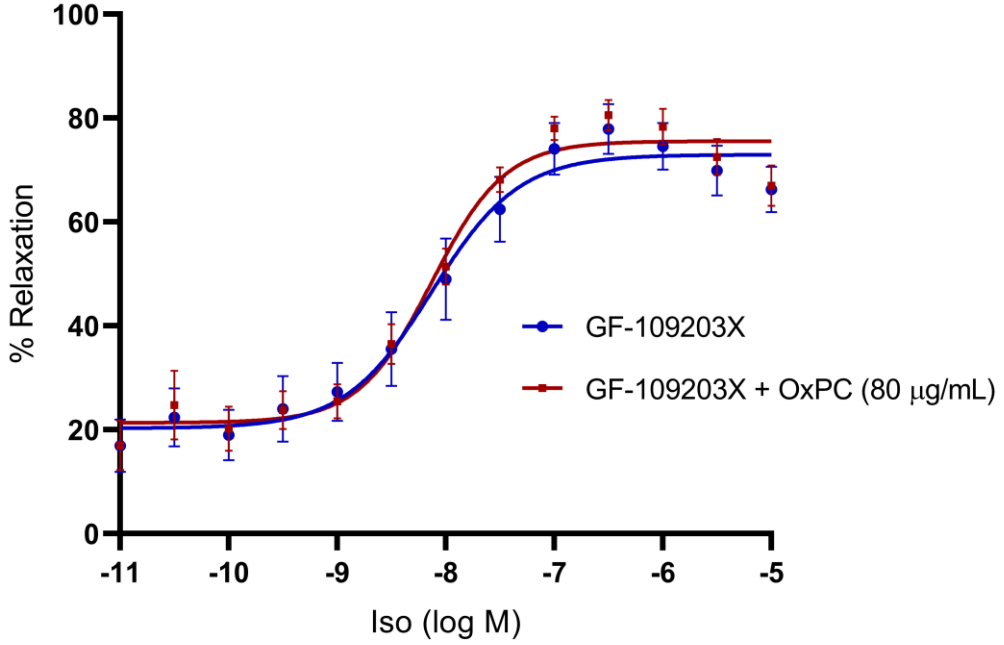
Figure 1A



Treatment	Isoproterenol (Iso) EC_{50}	P value
Control	3.3 nM	-
OxPC (80 μg/mL)	17.8 nM	<0.001

Figure 1B



Treatment	Isoproterenol (Iso) EC_{50}	P value
GF-109203X (10 μM)	7.5 nM	-
GF-109203X (10 μM) + OxPC (80 μg/mL)	7.4 nM	0.771