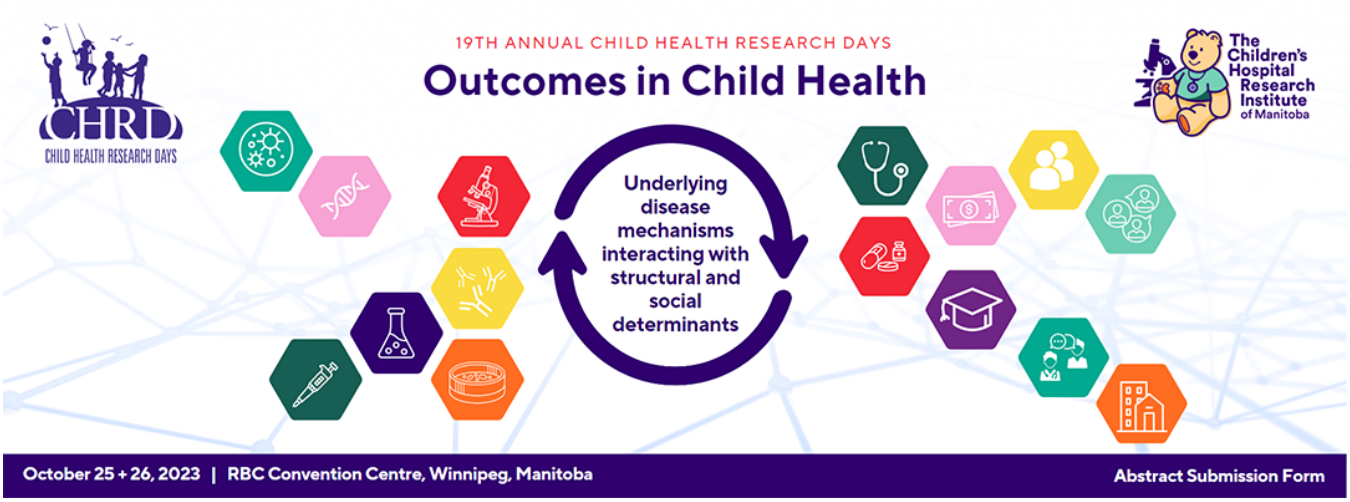

CHRD 2023: Abstract Submission Form

Submitter Name
Dheerendra Pandey

Presenter Name
Dheerendra Pandey

Presenter Status
PhD Student

Research Category
Basic Science

Role in the project
Perform Experiments

Title
TRPA1 in human airway fibroblasts: a role for mediating oxidized phosphatidylcholine pathobiology in asthma?

Background
Oxidative stress associated with allergic asthma generates bioactive pro-inflammatory mediators, including oxidized phosphatidylcholines (eg OxPAPC). We reported OxPAPC induces bronchial narrowing and intracellular Ca2+ in human airway smooth muscle cells via transient receptor potential ankyrin 1 (TRPA1). As human airway fibroblasts (HAF) contribute to asthma pathobiology, we screened TRPA1 expression, function and susceptibility to OxPAPC activation in HAFs.

Objective
please refere to uploaded file

Methods
We used qPCR and immunoblotting to assay TRPA1 abundance in primary HAF cultures from central bronchi of resected lung specimens of human donors (4 males, 5 females) undergoing lung surgery. We measured TRPA1-mediated Ca2+ influx in Fluo-4-loaded HAFs in response to TRPA1 agonist (allyl isothiocyanate (AITC, 0.1-3uM)) and OxPAPC (40 µg /mL), tracking change in fluorescence (F/Fo) with a Cytation 5 reader. Data were analyzed by one-way ANOVA, and Tukey's post hoc test.

Results
qPCR and immunoblotting showed all HAF lines express abundant TRPA1. This was corroborated by Ca2+ imaging: 0.1uM AITC induced a biphasic response, with a peak increase of

4.57±0.27 and an sustained plateau of 2.887±0.24 F/Fo. There were no sex-based differences in TRPA expression or function. Pearson correlation analysis showed significant association between TRPA1 expression and AITC-induced F/Fo. OxPAPC induces an increase in intracellular Ca2+, marked by a rapid rise to peak (F/Fo=1.42±0.005 at 28±4 seconds), and a sustained plateau (F/Fo=1.2±0.005) for up to 3 minutes.

Conclusion
HAF express TRPA1 that can be activated by OxPAPC, which accumulates in airways after allergic challenge. This suggests that OxPAPC could induce a role for HAF in asthma pathophysiology.

Table/Figure File
CHRD Research-Abstract_AH2023.pdf

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TRPA1 in human airway fibroblasts: a role for mediating oxidized phosphatidylcholine pathobiology in asthma?

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Rationale: Oxidative stress associated with allergic asthma generates bioactive pro-inflammatory mediators, including oxidized phosphatidylcholines (eg OxPAPC). We reported OxPAPC induces bronchial narrowing and intracellular Ca^{2+} in human airway smooth muscle cells via transient receptor potential ankyrin 1 (TRPA1). As human airway fibroblasts (HAF) contribute to asthma pathobiology, we screened TRPA1 expression, function and susceptibility to OxPAPC activation in HAFs.

Methods: We used qPCR and immunoblotting to assay TRPA1 abundance in primary HAF cultures from central bronchi of resected lung specimens of human donors (4 males, 5 females) undergoing lung surgery. We measured TRPA1-mediated Ca^{2+} influx in Fluo-4-loaded HAFs in response to TRPA1 agonist (allyl isothiocyanate (AITC, 0.1-3uM)) and OxPAPC (40 µg /mL), tracking change in fluorescence (F/Fo) with a Cytation 5 reader. Data were analyzed by one-way ANOVA, and Tukey's post hoc test.

Results: qPCR and immunoblotting showed all HAF lines express abundant TRPA1. This was corroborated by Ca^{2+} imaging: 0.1uM AITC induced a biphasic response, with a peak increase of 4.57±0.27 and an sustained plateau of 2.887±0.24 F/Fo. There were no sex-based differences in TRPA expression or function. Pearson correlation analysis showed significant association between TRPA1 expression and AITC-induced F/Fo. OxPAPC induces an increase in intracellular Ca^{2+}, marked by a rapid rise to peak (F/Fo=1.42±0.005 at 28±4 seconds), and a sustained plateau (F/Fo=1.2±0.005) for up to 3 minutes.

Conclusion: HAF express TRPA1 that can be activated by OxPAPC, which accumulates in airways after allergic challenge. This suggests that OxPAPC could induce a role for HAF in asthma pathophysiology.