Oxidized Phosphatidylcholine Causes Airway Narrowing: Novel Indication for Airway Hyperresponsiveness in Asthma

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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 (OxPAPCs) are pro-inflammatory and accumulate in the lungs of mice and humans after inhaled allergen challenge.

• We have shown that OxPAPCs induce intracellular Ca2+ flux in human airway smooth muscle cells (Figure 1), and this triggers muscle contraction that leads to airway narrowing, the principal event in asthma attacks.

• Here, we test the hypothesis that OxPAPCs cause airway narrowing under control of pathways that regulate cytoplasmic Ca2+ flux in human airway smooth muscle.

Materials & Methods

• Murine thin-cut lung slices (TCLS) were obtained using a vibratome, creating about ~180-µm-thick lung slices that were cultured for up to 96 hours for experiments.

• Phase-contrast video microscopy was used to assess airway narrowing, with real-time changes in airway lumen area recorded for 3 min, after exposure to OxPAPC (i.e., oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine, 80 µg/ml). PSPC (1-palmitoyl-2-stearoyl-sn-glycero-3-phosphocholine, 80 µg/ml, lipid control) or methacholine (mCh, 0.1 µM, positive control) in the presence and absence of extracellular Ca2+.

• To determine the role of intracellular Ca2+ stores, TCLS were pretreated with ryanodine channel inhibitors (ryanodine 100 µM).

• NIH/Scion Image J software was used to determine changes in airway lumen area.

Results

• Extracellular Ca2+ required for maximum airway narrowing induced by OxPAPC (Murine TCLS)

• Ryanodine inhibits OxPAPC-induced airway narrowing

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Conclusion

• OxPAPC (80µg/mL) induced a significant 15% airway closure, compared to non-oxidized phosphatidylcholine (this would increase airflow resistance by 4 times).

• In the absence of extracellular Ca2+, OxPAPC did not induce any airway narrowing indicating Ca2+ influx is required for contraction (Figure 9).

• Ryanodine receptor inhibition (i.e., ryanodine 100µM, in media with extracellular Ca2+) completely abrogated OxPAPC-induced airway narrowing, indicating that the primary source of intracellular Ca2+ release is via ryanodine receptors (Figure 9).

• These findings demonstrate that OxPAPC’s mediate airway narrowing via influx of Ca2+ from both the extracellular source and the ryanodine receptor regulated stores of the sarcoplasmic reticulum. This implicates a role of OxPAPCs in airway hyperresponsiveness, a hallmark feature of asthma.

• No current therapies target OxPAPC effects, therefore it is important to continue to elucidate the mechanism by which OxPAPC causes smooth muscle contraction and airway narrowing, as this could lead to new therapeutic development options.

References


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