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 Ca^{2+} 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 Ca^{2+} flux in human airway smooth muscle.***

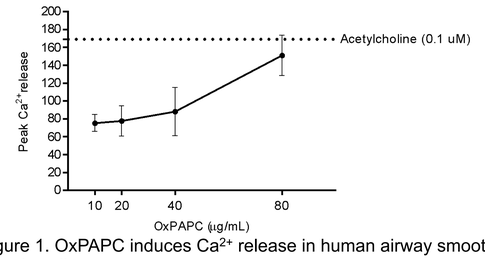

Figure 1. OxPAPC induces Ca^{2+} release in human airway smooth muscle cells

Materials & Methods

- Murine thin-cut lung slices (**TCLS**) were obtained using a vibratome, creating about ~180 um 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 **Mch** (methacholine, 0.1 µM, positive control) in the presence and absence of extracellular Ca^{2+}.
- To determine the role of intracellular Ca^{2+} stores, TCLS were pre-treated with ryanodine channel inhibitors (ryanodine 100 µM).
- NIH/Scion Image J software was used to determine changes in airway lumen area.

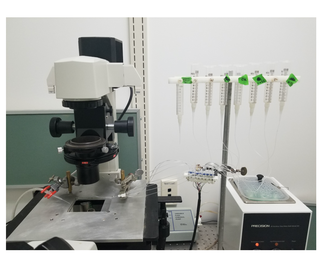

Figure 2. Set-up of experiment showing the phase-contrast microscope and the solenoid valve system for controlling solution flow

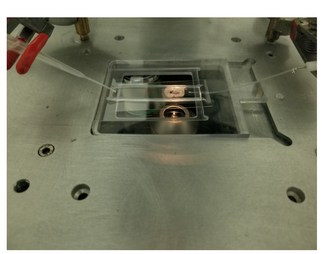

Figure 3. Perfusion chamber showing the prepared slide containing the thin-cut lung slice, the perfusion line, and vacuum line

Results

Extracellular Ca^{2+} Required for Maximum Airway Narrowing induced by OxPAPC (Murine TCLS)

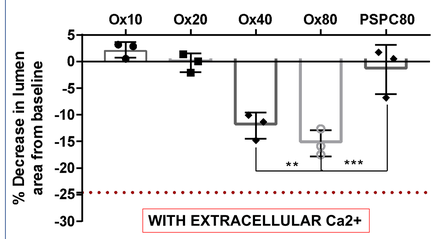

Figure 4. Airway narrowing induced by increasing concentrations of OxPAPC, compared to PSPC 80ug/ml in the presence of extracellular Ca^{2+}

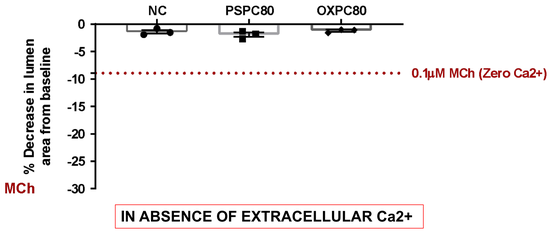

Figure 5. Airway Narrowing induced by negative control (HEPES), PSPC 80ug/ml, and OxPAPC 80ug/ml under Ca^{2+} free conditions

Ryanodine Inhibits OxPAPC-Induced Airway Narrowing

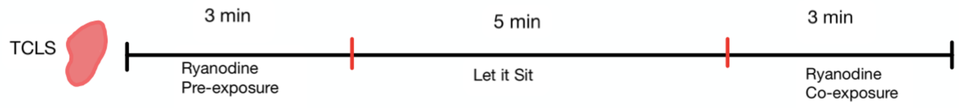

Figure 6. Timeline for TCLS experiment with ryanodine receptor inhibition

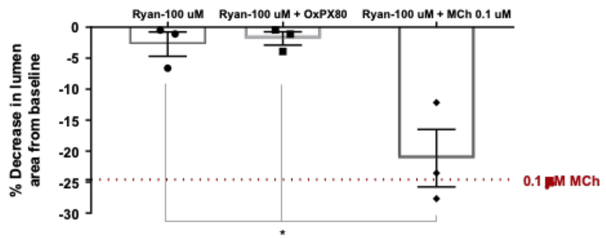

Figure 7. Airway Narrowing induced by ryanodine 100uM, ryanodine 100uM + OxPAPC 80ug/ml co-exposure, and ryanodine 100uM + Mch 0.1 uM co-exposure.

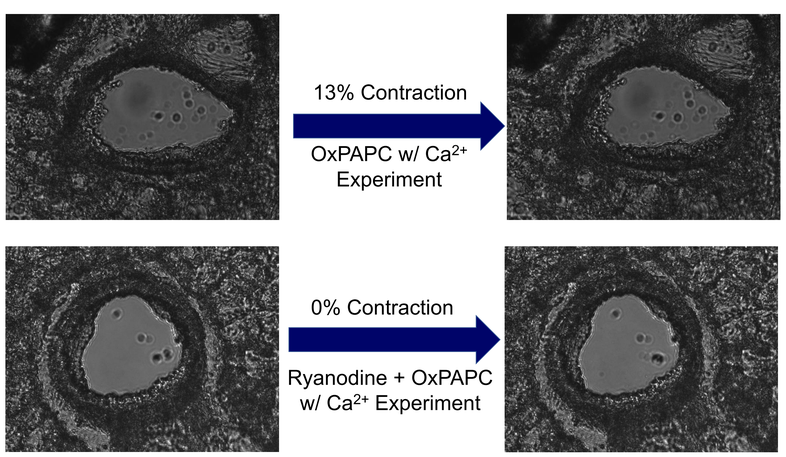

Figure 8. Experimental images show OxPAPC-induced contraction and its inhibition by ryanodine (this is consistent with our prior observation that ryanodine inhibits Ca^{2+} flux induced by OxPAPC in human airway smooth muscle cells)

Conclusion

- OxPAPC (80ug/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 Ca^{2+}, OxPAPC did not induce any airway narrowing indicating Ca^{2+} influx is required for contraction (Figure 9)
- Ryanodine receptor inhibition (i.e.. ryanodine 100uM, in media with extracellular Ca^{2+}) completely abrogated OxPAPC-induced airway narrowing, indicating that the primary source of intracellular Ca^{2+} release is via ryanodine receptors (Figure 9).
- ***These findings demonstrate that OxPAPC's mediate airway narrowing via flux of Ca^{2+} from both the extracellular source and the ryanodine receptor regulated stores of the sarcoplasmic reticulum. This implicates a role of OxPAPC's 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.

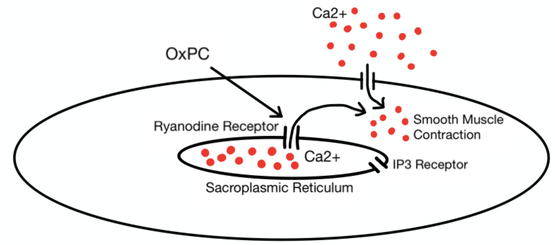

Figure 9. Schematic showing the effects of OxPAPC on a smooth muscle cell

References

1. Pascoe, C. D., Jha, A., Ryu, M. H., Ragheb, M., Vaghasiya, J., Basu, S., Stelmack, G. L., Srinathan, S., Kidane, B., Kindrachuk, J., O'Byrne, P. M., Gauvreau, G. M., Ravandi, A., Carlsten, C., Halayko, A. J., & Canadian Respiratory Research Network (2020). Allergen inhalation generates pro-inflammatory oxidised phosphatidylcholine associated with airway dysfunction. *The European respiratory journal*, 2000839. Advance online publication. https://doi.org/10.1183/13993003.00839-2020

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