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Oxidized phosphatidylcholine induces mitochondrial and endoplasmic reticulum stress in airway epithelial cells

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Background:

The lung is rich in phosphatidylcholines (PCs) which are susceptible to oxidative damage, generating bioactive PCs including oxidized 2-arachidonoyl-1-palmitoyl-sn-glycero-3-phosphocholine (OxPAPC). Our lab has identified the presence of OxPAPC in lung lavage from people with asthma undergoing allergen challenge and have characterized the ability of OxPAPC to impair normal epithelial barrier function. OxPAPC could promote airway dysfunction in asthma but the pathological mechanisms are currently unknown which limits our ability to successfully target them for therapeutic benefit.

Objective:

Oxidized phosphatidylcholine impairs mitochondrial function and induces a state of cellular stress in airway epithelial cells.

Methods:

The human airway epithelial cell line, Calu3, was incubated with OxPAPC (0-160 μg/mL) for 2 hours and the level of reactive oxygen species (ROS) in the cell was measured using fluorescent dyes. Mitochondrial function was assessed using a proxy for mitochondrial membrane potential as well as mitochondrial function within an Agilent Seahorse Metabolic Analyzer. After 24 hours of OxPAPC exposure, RNA and protein markers for endoplasmic reticulum (ER) stress were measured. Experiments were completed in at least triplicate.

Results:

OxPAPC dose dependently increase the abundance of ROS compared to control, exhibiting a 6-fold increase in DCF staining after 2 hours at 160 μg/mL OxPAPC ($p<0.0001$). OxPAPC also dose dependently induce mitochondrial dysfunction, as evidenced by increased mitochondrial membrane potential (2-fold increase at 160 μg/mL OxPAPC; $p<0.0001$), and by significant impairment in each of basal respiration, ATP production, and maximal respiration rate ($p<0.01$). Surprisingly, OxPAPC doses as low as 10 μg/mL induce significant metabolic dysfunction. Finally, OxPAPC exposure results in significant increases in markers of ER stress including CHOP, ATF4, and spliced xBP1 ($p<0.05$).

Conclusion:

OxPAPC induces comprehensive cellular and metabolic dysfunction in epithelial cells which could explain their barrier disruptive properties. OxPAPC is an untargeted feature of asthma that could drive key pathophysiologic changes in asthma.