The Effect of Oxidized Phosphatidylcholines on CD38 Abundance in Human Airway Smooth Muscle Cells

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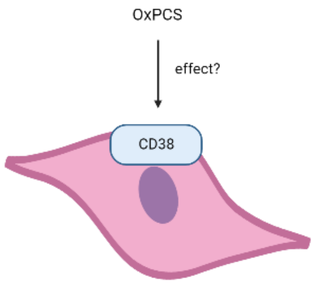
INTRODUCTION

Asthma is a chronic, respiratory disease that affects 12% of all children in Canada, and is the number one cause of childhood Emergency room visits . A defining disease trait is the hypercontractility of the airway smooth muscles (ASM). This is mediated by intracellular Ca^{2+}flux, including the effects of a transmembrane cyclic ADP ribose hydrolase, CD38, which is increased in ASM cells from asthmatics. Previous work in our lab showed that allergen challenge in humans and mice generated oxidized phosphatidylcholines (OxPCs) in the airways. Acute exposure of smooth muscle cells to OxPC is sufficient to induce contraction and airway narrowing. Thus, we sought to explore the effect of prolonged exposure on CD38 abundance in HASM cells.

Figure 1: Normal Human Airway v Asthmatic Human Airway

Hypothesis

Does prolonged OxPC exposure affect CD38 abundance in HASM cells?

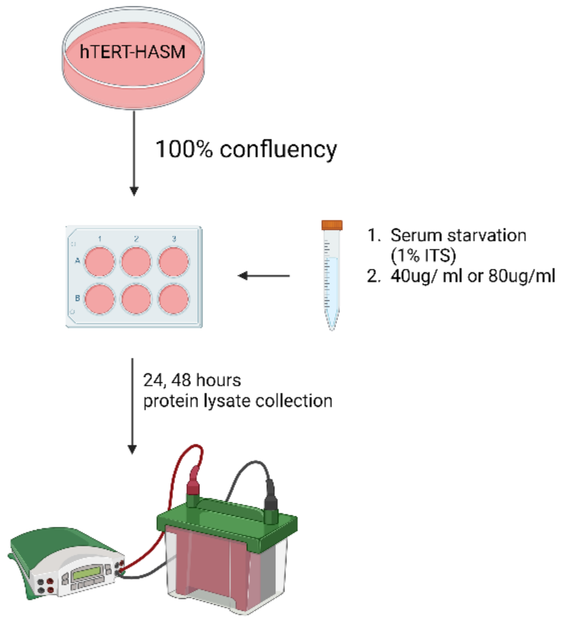


AIMS

- Assess the impact of OxPCs on CD38 abundance in HASM cells.
- Determine if there is a difference in abundance between concentrations of OxPCs.
- Determine if there is a difference in abundance between exposure times.

METHODS

We used human telomerase reverse transcriptase (hTERT) HASM cell lines, generated from primary cell culture of bronchial smooth muscle tissue obtained from human participants who exhibited normal airway function (N=5). hTERT-HASM cells were pretreated with OxPC (40ug/mL or 80ug/mL) for 24 and 48 hours. immunoblotting and quantitative densitometry to was used to assess the abundance of CD38. Data were analyzed by one-way ANOVA and Dunnett test.



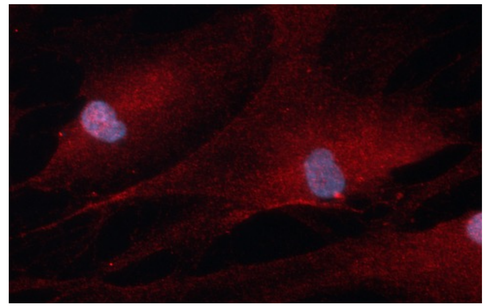


Figure 2: Verification of CD38s presence in hTERT HASM cells via Epi-fluorescence microscopy

Results

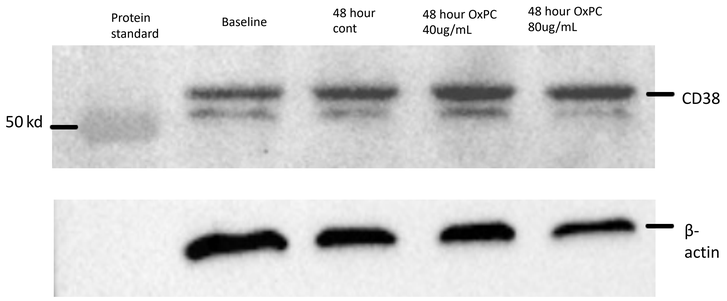


Figure 3: representative western blot of CD38 abundance in hTERT-HASM 3283 (N=1), with β-actin loading control.

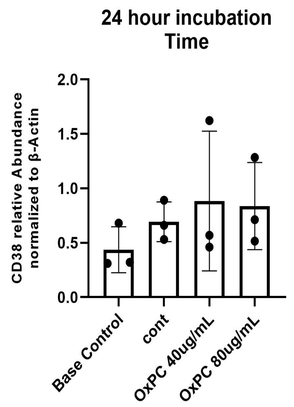


Figure 4: relative abundance of cd38 after 24 hours OxPC exposure. Base control=0-hour, cont=time matched

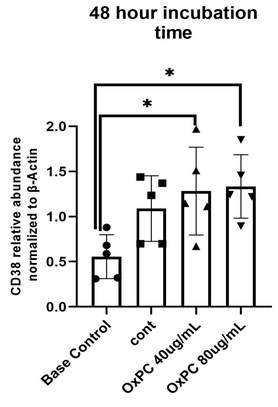


Figure 5: relative abundance of cd38 after 48 hours OxPC exposure. *p<0..05 Base control=0-hour, cont=time matched

After 24 hours OxPC exposure (40ug/mL or 80ug/mL), CD38 abundance increased 27% and 20%, respectively, when compared to time matched control cultures (N=3) (figure 3). In the 48-hour group, we measured an increase of 18% and 23%, respectively, when compared to the time matched control (N=5)(figure 4). Further elucidation is required to evaluate the effect that the serum starvation had on the abundance of CD38 as in both groups we saw large increases, with the 48-hour groups seeing statistically significant differences Likely due to the limited number of replicates we were able to achieve, statistically significant differences in CD38 abundance were not evident between the time matched controls and the OxPC treated HASM cells, however, but as noted above, there was a distinct upward trend.

Conclusion

- Prolonged exposure to OxPCs may increase CD38 abundance in HASM cells
- differences between individual cell lines make it difficult to detect a statistically significant change
- The biological relevance of OxPC effect on CD38, and its role in asthma pathophysiology needs further elucidation.

ACKNOWLEDGEMENTS