The effects of flavoured electronic cigarette liquid on calcium signalling and ion transport function of airway epithelial cell

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Introduction

E-Liquid cigarettes are becoming popular especially among the young people. They work by using a battery powered device to deliver aerosolised nicotine considered to be less harmful than conventional cigarettes(1). However, there has been recent evidence that exposing select flavours of e-liquid leads to cytotoxicity in epithelial cells and disruption of Calcium (Ca^{2+}) signaling(2)

Ca^{2+} signalling is an important biological process that regulates many aspects of cellular function. Intracellular calcium levels are kept low so a rise in Ca^{2+} levels allows for communication to take place. Therefore disruption in this process can lead to abnormal inflammatory responses and ion channel activity

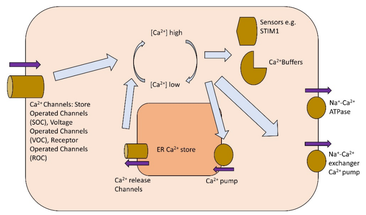

Figure 1: Ca^{2+} signalling overview. Purple arrows indicate movement of Ca^{2+}. ER: Endoplasmic reticulum

- Depleted Ca^{2+} stores sensed by sensors like STIM1 in the Endoplasmic reticulum (ER) lumen can trigger store operated Ca^{2+} entry (SOCE) so that the stores can be refilled with Ca^{2+}.

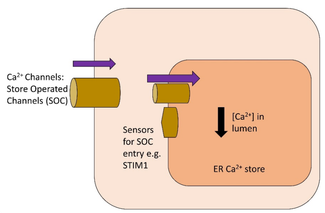

Figure 2: SOCE:- when the ER stores are depleted, sensors like STIM1 causes Store Operated Ca^{2+} channels to be activated and initiate SOCE. SOC: Store Operated Channels, ER: Endoplasmic reticulum

Aims

Our main aim of the project was to investigate how the intracellular Ca^{2+} signaling in human airway epithelial cells is affected when exposed to banana pudding flavour of e-liquid, and study the contribution of the entry of calcium to the change in calcium levels caused by exposure to e-liquid.

Methods

- Cell Culture: Calu-3 cells were cultured in a laminar flow hood.

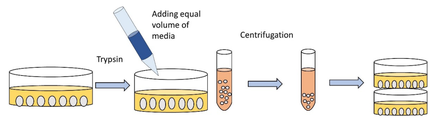

Figure 3: Steps involved in cell culture

- Measuring Ca^{2+} Fluorescence: Ca^{2+} indicator and a photometer were used to measure intracellular Ca^{2+} levels. Intracellular Ca^{2+} changes were observed by doing baseline measurement in high Ca^{2+} solution, then adding banana flavoured e-liquid in high Ca^{2+} and 0 Ca^{2+}. The control experiments involved the "vehicle" PG/VG which is used to dissolve the e-liquid flavouring.
- Ussing Chamber: This was used to learn about the ion channel function in polarised cells. After adding e-liquid and PG/VG, Cystic Fibrosis Transmembrane conductance Regulator (CFTR)-inhibitor 172 was added to investigate the effect of e-liquid on CFTR function.

Results

Addition of banana-pudding flavoured e-liquid elevated intracellular calcium in Calu-3 cells:

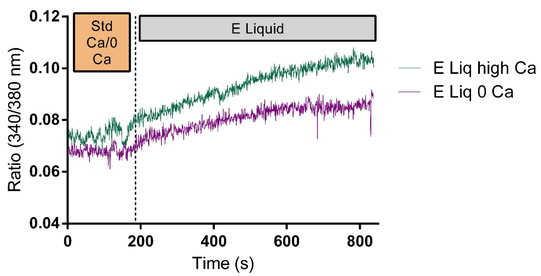

Figure 4: Sample traces of the effect of adding e-liquid in high Ca^{2+} solution and e-liquid in 0 Ca^{2+} solution.

Perfusion of cells with E-liquid in high Ca^{2+} solution and 0 Ca^{2+} solution evoked different responses.

Results

Perfusion with high Ca^{2+} solution after exposure to E-liquid in 0 Ca^{2+} solution:

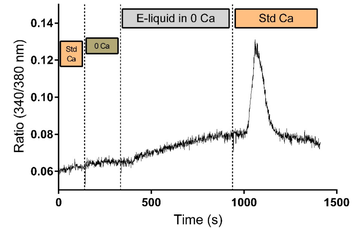

Figure 5: Sample trace showing the effect of e-liquid in 0 Ca^{2+}.

Addition of e-liquid in high Ca^{2+} and 0 Ca^{2+}:

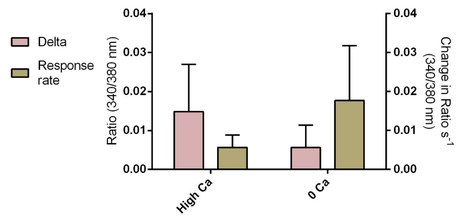

Figure 6: Responses of e-liquid in high Ca^{2+} and 0 Ca^{2+}. P-value for delta = 0.0163 response rate = 0.0240.

E-liquid in high Ca^{2+} showed a bigger change in ratio than in 0 Ca^{2+} while the response rate was slower for high Ca^{2+} than 0 Ca^{2+}

SOCE in e-liquid and PG/VG:

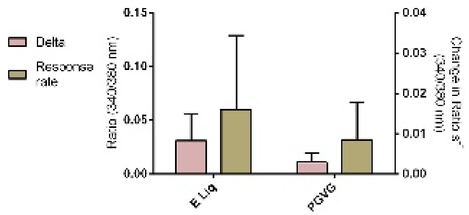

Figure 7: Effect of adding calcium after exposure to e-liquid and PG/VG in 0 Ca^{2+}. Delta and response rate of SOCE. P-values for delta = 0.1239, response rate = 0.5541.

E-liquid showed a faster and greater increase in Ca^{2+} concentration than PG/VG.

Results

Adding e-liquid, PG/VG and CFTR-inhibitor in the Ussing Chamber experiment:

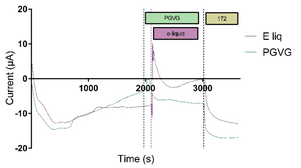

Figure 7: Sample trace for Ussing chamber experiment.

E-liquid caused a bigger change in current than PG/VG, while the decrease in current for both after adding the inhibitor was similar.

Discussion

- Big response in the presence of high Ca^{2+} shows there is a component of Ca^{2+} influx, while e-liquid response in 0 Ca^{2+} shows that there is also the component of store release.
- SOCE is more pronounced with e-liquid than PG/VG, suggesting the e-liquid flavouring causes store release, which could lead to a variety of responses such as cell proliferation, inflammation etc.
- E-liquid impacts CFTR function but this will need to be further investigated.

Acknowldegments

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References

1. Muthumalage T, Prinz M, Ansah KO, Gerloff J, Sundar IK, Rahman I. Inflammatory and Oxidative Responses Induced by Exposure to Commonly Used e-Cigarette Flavoring Chemicals and Flavored e-Liquids without Nicotine. Frontiers in Physiology. 2018;8(1130).
2. Rowell TR, Reeber SL, Lee SL, Harris RA, Nethery RC, Herring AH, et al. Flavored e-cigarette liquids reduce proliferation and viability in the CALU3 airway epithelial cell line. American Journal of Physiology - Lung Cellular and Molecular Physiology. 2017;313(1):L52-L66.