Does Gastro-oesophageal Reflux Contribute to Cystic Fibrosis Lung Disease?

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Introduction

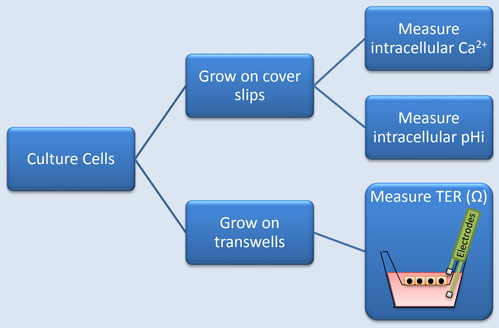
Cystic Fibrosis (CF) is an autosomal recessive disease which affects multiple organs in the body, including the lungs. The most common mutation in CF is deltaF508 [1]. This mutation causes the CF Transmembrane Conductance Regulator (CFTR) to be absent from the plasma membrane [2]. CFTR is an epithelial Cl^- channel, so if it is absent, there is less Cl^- transport across epithelial cells [3]. Gastro-oesophageal reflux disease (GERD) is 6-8 times more likely to occur in CF patients than in healthy individuals. If a CF patient aspirates reflux agents into their lungs, this may contribute to their lung disease. There is a link between CF patients who suffer from GERD and the need for a bilateral lung transplant, and 5 years post transplant only yields 50-60% survival [4]. If more is known about the effects of aspirated reflux agents in CF then it may be possible to develop treatments that help prevent , or delay, a bilateral lung transplant, thereby increasing CF patient survival time.

Aims

To investigate whether reflux agents have a detrimental effect on lung epithelial cells, and to assess whether there is a significant difference between non CF (WT) and CF cells. The following physiological parameters were measured in response to potential reflux agents; bile acid (BA), acid, and pepsin:

- Barrier function
- Intracellular calcium
- Intracellular pH

Methods



CFBE (CF bronchial epithelial) cell lines were used. CFBE.WT express CFTR whereas CFBE.ΔF express deltaF508 CFTR [5]. Both cell line were transferred to cover slips and then used after 2 days, and both cell lines were grown on transwells; their transepithelial resistance (TER) was measured daily until a stable TER was reached. The cover slips were loaded with a Ca^{2+} sensitive dye and then placed under the lens of an epi-fluorescent microscope. The cells were then perfused with the reflux agents whilst being excited at 2 respective wavelengths. Intracellular pH (pHi) was measured in a similar way to Ca^{2+} but instead using a pH sensitive dye. TER was measured using EVOM chopstick electrodes.

Results - Effect of 100μM BA on intracellular Ca^{2+} and TER

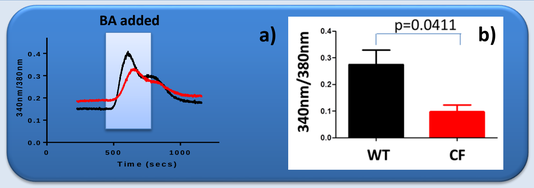


Figure 1: a) example Ca^{2+} trace to show the effect of 100μM BA on WT and CF cells. **b)** the increase in intracellular Ca^{2+} is greater in WT cells compared to CF cells

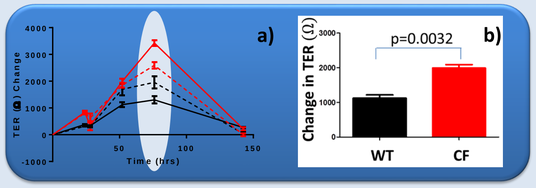


Figure 2: a) TER (Ω) trace to show the change in TER after 48hrs exposure to 100μM BA on WT. Dotted lines show TER change in absence of BA. **b)** the TER is greater in CF cells compared to WT cells, however, both are reversible after washout.

Results - Effect of Acid pH6 on pHi and TER

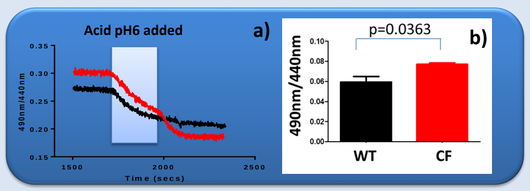


Figure 3: a) example pHi trace to show the effect of acid (pH6) on intracellular pH on WT and CF cells. **b)** the increase in acidification is greater in CF cells compared to WT cells

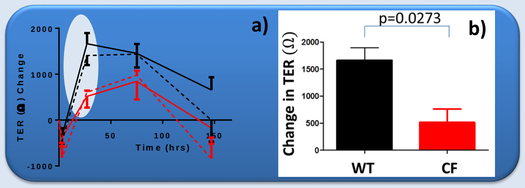


Figure 4: a) TER (Ω) trace to show the change in TER after 24hrs exposure to acid (pH6) on WT and CF. Dotted lines show TER change in absence of acid. **b)** the TER is greater in WT cells compared to CF cells, however, both are reversible after washout.

Results - Effect of Acid + Pepsin on TER

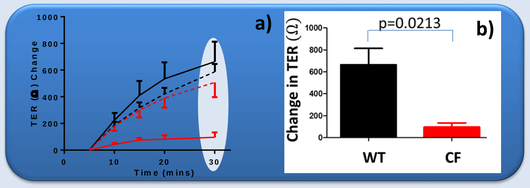


Figure 5: a) TER (Ω) trace to show the change in TER after 30min exposure to acid (pH6) + pepsin on WT and CF cells. Dotted lines show TER change in absence of acid + pepsin. **b)** the TER is greater in WT cells compared to CF cells, however, both are reversible after washout.

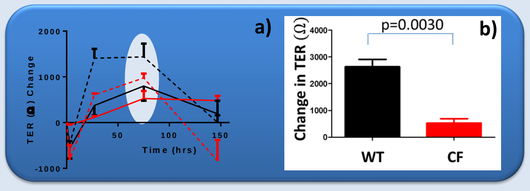


Figure 6: a) TER (Ω) trace to show the change in TER after 48hrs exposure to acid (pH6) + pepsin on WT and CF. Dotted lines show TER change in absence of acid + pepsin. **b)** the TER is greater in WT cells compared to CF cells, however, both are reversible after washout.

Discussion/Conclusions

- Exposure to 100μM BA caused an increase in intracellular Ca^{2+}, but this response was blunted in CF cells. Since an increase in intracellular Ca^{2+} is known to stimulate airway cells to secrete more salt and fluid as a protective mechanism against noxious agents, this could be a factor contributing to the pathology of CF lung disease.
- A decrease in TER in CF cells in response to acid and acid + pepsin means that barrier function has been impaired, therefore CF patient lungs may be more vulnerable to disease causing agents.
- The decrease in pH in response to acid is significantly larger in CF cells than in WT cells. This suggests that CF patients could be more severely affected than non CF patients if acid was to be aspirated into the lungs.
- From my findings, I can conclude that the reflux agents do have an effect on barrier function and intracellular ions. However, further research needs to be undertaken to fully conclude whether or not gastro-oesophageal reflux does contribute to CF lung disease.

References

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