A Potential Role of Epithelial Mesenchymal Transition in Aspiration Injury of the Lung

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Introduction

Gastric aspiration (the movement of gastric juice up the gullet and into the lungs) can damage the oesophagus. It is also believed to cause damage to the airways and is increasingly implicated as a risk factor in the chronic rejection of lung transplants.

Epithelial Mesenchymal Transition (EMT) is the process by which epithelial cells lose adhesion to other cells and/or their basement membrane, lose cell polarity and then transform into a different cell type, called a fibroblast. As a fibroblast, they can damage the airways by creating collagen deposits within the airway, narrowing it and therefore reducing ventilation.

EMT is suggested to be brought on by damage to the epithelial cell layer and the presence of certain pro-EMT factors, such as the enzyme MMP-9.

Pepsin (a stomach enzyme) and bile acids, can both be present in gastric aspirations, and potentially could cause damage to the airway epithelial layer, possibly leading to EMT.

Aims

- Measure the potential of pepsin and bile acids to damage human bronchial epithelial cells
- Find whether MMP-9, a marker for EMT, is released by damaged epithelial cells
- Use two different human bronchial epithelial cell lines in the experiments, observing any differences

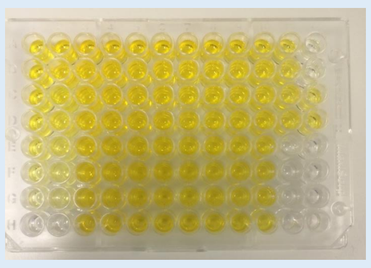
Methods

Cell lines: Two different cells lines were used: one named 16HBE14o- and the second called CFBE. The CFBE cells were then sub divided into two types, ones which were genetically engineered to be Wild Type (normal) cells, and the second to have the defect of the most common form of cystic fibrosis, called **Δ**F-508. Using Ussing Chambers the CFBE cell transport properties were measured to determine whether the CFBE cells had been successfully engineered into the two types.

Challenges: The two cell lines were both grown in 24-well plates then challenged by giving them solutions containing varying concentrations of Pepsin, or the bile acids Lithocholic Acid (LCA) and Chenodeoxycholic Acid (CA), and leaving them for 48 hours. The cell viability (percentage of the cells killed by the challenge) was measured using Cell TitreBlue.

ELISA: To measure MMP-9, one 24-well plate containing 16HBE14o- cells had its solution harvested after a CA challenge. The solution from the wells was then run through an ELISA plate to find any MMP-9. ELISA plates work by capturing the specific substance you are looking for (such as MMP-9) into the wells of the plate.

24-Well plate after a challenge. The colour is from the use of Cell TitreBlue. This chemical indicates the level of alive cells in each well, with dark blue representing 100% dead.

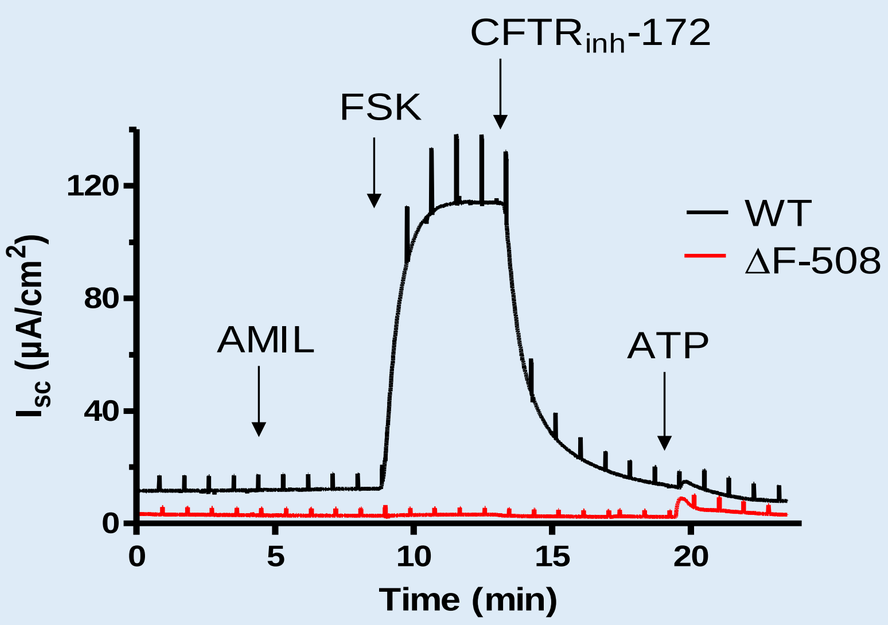


ELISA plate. The shade of yellow corresponds to the concertation of MMP-9 in the well.

Ussing Chambers

With Ussing Chambers it was proved that the CFBE cells were displaying the correct properties of their Wild Type and **Δ**F-508 subtypes. The difference in their lines (Fig 1) is due to the fully functioning CFTR protein in Wild Type reacting to the chemicals delivered to the cells in the Ussing Chamber, which changes the cells' ion transport properties and therefore the Short Circuit Current (I_{SC}) needed to clamp the cells at 0 Volts. The **Δ**F-508 line is mostly horizontal due to the CFTR protein being incorrectly expressed by the cell, so the effects of the chemicals are greatly reduced.

Figure 1:



MMP-9 ELISA with Challenged 16HBE14o- Cells

The ELISA results showed that there was MMP-9 present in the solution (Fig 2), the control had the least, and that there was significantly more in some of the groups compared to the control.

This suggests that the cells could be releasing increased levels of MMP-9 in response to the bile acid. However, the results do not seem to show a trend between acid concentration and MMP-9 release.

The normalised MMP-9 graph (Fig 3) is derived from the data in Fig 2. It represents the MMP-9 levels measured after challenge corrected for cell viability. The MMP-9 rises, due to normalising, most notably at the 100uM and 75uM CA concentrations - strengthening the theory that the MMP-9 release is in response to cell damage.

The presence of MMP-9 hints that undergoing EMT may be a distinct possibility for some of these cells.

Figure 2:

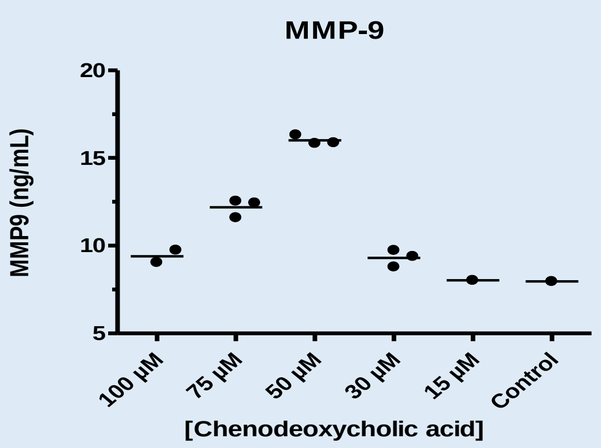
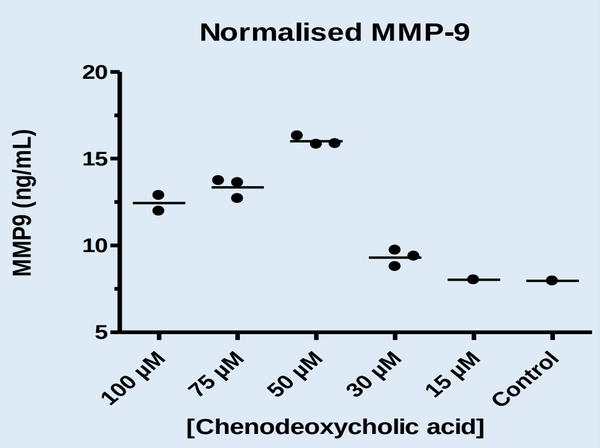


Figure 3:



Acid Challenge Cell Viability

After being challenged with bile acids (Fig 4 & Fig 5), the 16HBE14o- were dying, and there seemed to be a rise in % dead as the acid concentration rose. There was significantly more dead cells at the highest concentration, of both acids, compared to their controls.

Lithocholic acid appeared to be more lethal than Chenodeoxycholic acid, even though a lower concentration range was used. At the lower ranges, neither acid appeared to be killing the cells.

Figure 4:

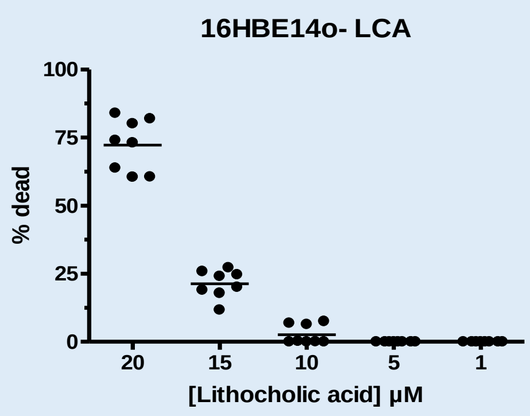
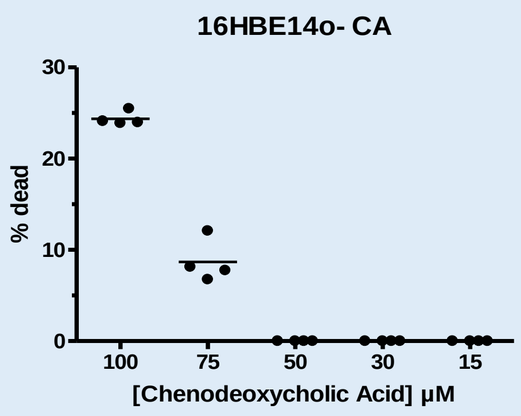


Figure 5:



CFBE Challenges

The resting solution that the cells were left in before being challenged with pepsin or acid was found to be deadly to CFBE cells after quite a short length of time. This meant that a very large number of CFBE cells died regardless of the challenge made to them. Consequently, no suitable data could be obtained from the challenges done to these cells.

Pepsin Challenges

Challenges done using pepsin failed to cause any notable cell death, even at the higher concentrations.

Pepsin works best in acidic conditions, whereas the challenge solution was at a neutral pH, so the lack of damage was likely due to the neutral pH inactivating the pepsin.

Conclusions/Discussion

The research shows promise that gastric aspiration may cause EMT and lung tissue damage, since bile acids were damaging the lung bronchial epithelial cells and this damage seemed to be causing an increased release of the pro-EMT factor MMP-9.

However, due to the small number of repeats used for each experiment, these results can only be seen as a suggestion of what happens. Even some of the quite expected results, such as acid damaging the cells, would require more testing to confirm.