The effect of Reactive Oxygen Species on innate defence mechanisms of normal & cystic fibrosis airway epithelial cells

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Background

- Cystic Fibrosis (CF) is a genetic disease caused by a **mutation** in the gene called **CFTR**, which encodes an **ion channel** on the apical side of the **airway epithelial cells**. CFTR plays an important role in **anion, especially chloride, Cl- and bicarbonate, HCO_3-, secretion** from epithelial cells (Figure 1).
- People with CF usually suffer from **repeated bacterial infections**, hence **lung inflammation**, due to **viscous and congested mucus** in the airways. It is their enhanced susceptibility to infections that shortens their lives.
- During inflammation, **reactive oxygen species (ROS)**, such as hydrogen peroxide, H_2O_2, are produced, by **neutrophils**, as a **natural immunity to kill the pathogens**.
- Epithelial cells line the inner surface of the airways that is in contact with the air. The epithelium is covered and protected by the **airway surface liquid (ASL)**. ASL serves as a **defence barrier**, through both **physical and chemical mechanisms**, to keep the epithelium free from infections (Figure 2).
- **Depth and pH of the ASL are tightly regulated** to maintain efficient mucus clearance and function of the antimicrobials (Figure 2). **ASL pH can be altered by changes in HCO_3- transport** from epithelial cells. A **normal ASL** should have pH of around **7.4**. In CF, defective anion secretion leads to **ASL dehydration and acidity** (pH < 7,0)
- Studies have suggested that **ROS can affect both physical and chemical defence mechanisms** of the airway epithelial cells.

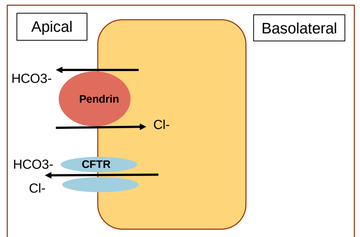

Figure 1. Illustration of an airway epithelial cell and its ion channels and transporters involved in anion secretion on the apical side. CFTR is an anion channel that secretes Cl- and HCO_3-. Pendrin is a Cl-/HCO_3- exchanger.

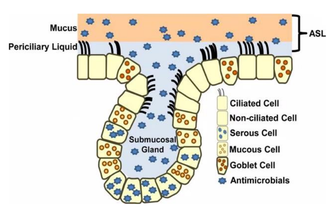

Figure 2. Diagram showing the epithelium of a submucosal gland in the airway and its associated ASL layer. Mucous and goblet cells produce mucus which traps pathogens and can be swept away by movement of the cilia. Serous cells release antimicrobials to kill pathogens[1].

Methods

- **Tissue Culture**: Two Calu-3 cell lines: one cell line has high expression level of CFTR channels (Calu-3 ALTER), the other cell line has CFTR gene removed (Calu-3 KO); were grown separately in supplemented media on semi-permeable supports under the same conditions: 37ºC, 5% CO_2.
- **ASL pH**: ASL was stained with a pH-sensitive fluorescent dye that is cell impermeant overnight at 37°C, 5% CO_2. pH was estimated using highly buffered solutions of known pH in order to provide a standard curve. ASL pH were monitored in real time using an automated plate reader containing a temperature and a CO_2 module.
- **HCO_3- transporters activity**: assessed by real-time intracellular pH (pHi) measurements.
- **Epithelial integrity**: assessed by transepithelial electrical resistance (TEER) measurements of the cultured monolayers using standard chopstick electrodes.

Aim

To investigate the **effects** of *tert*-butyl hydroperoxide (**t-BOOH)**, as a **ROS**, on **epithelial integrity** and on **regulation of ASL pH via HCO_3- transport** in **CF** and **non-CF airways**.

Results

- **ASL pH**: t-BOOH 0.3 mM did not show any significant effects on ASL pH but t-BOOH 1 mM caused a marked increase in ASL pH. There was not a significant difference between the effects on the two cell lines (**Figure 3**)
- **Intracellular pH (pHi)**: Exposure to t-BOOH on the apical side caused a marked decrease in pHi. There was very small increase in pHi when the monolayers were exposed to zero chloride (0Cl-) condition; but there was a marked increase in pHi when they were exposed to 0Cl- condition with t-BOOH (0.3 mM). pHi recovered when these conditions were removed. The differences between two cell lines were not significant (**Figure 4 & 5**).
- **TEER measurement**: TEER measurements of monolayers both cell lines decreased after 48 hours of exposure to t-BOOH (0.3 mM), either on apical, basolateral or both sides. And they all decreased at a faster rate than that of the control (**Figure 6**).

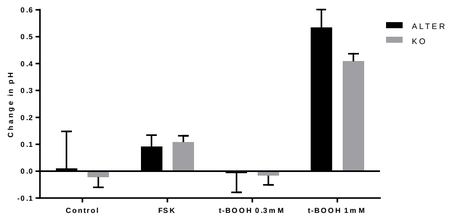

Figure 3. Mean differences in ASL pH of ALTER and KO monolayers after being exposed overnight to t-BOOH 0.3 mM (low concentration), t-BOOH 1mM (higher concentration), Forskolin 10uM (positive control) and DMSO (negative control).

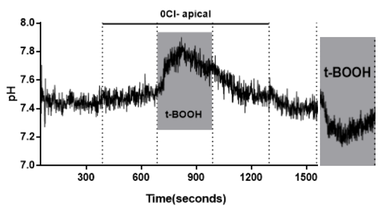

Figure 4. An example trace of real-time changes in intracellular pH when monolayer of Calu-3 cells is exposed apically to: first Krebs solution (high Cl-); 0Cl-; 0Cl- with t-BOOH; then back to 0Cl-; and back to high Cl- conditions; also t-BOOH only.

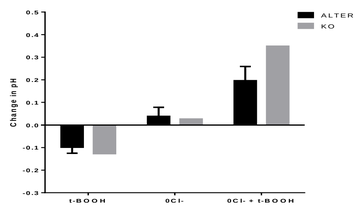

Figure 5. Mean differences in intracellular pH when monolayers of ALTER and KO cells are subjected to t-BOOH only; 0Cl- only and 0Cl- with t-BOOH.

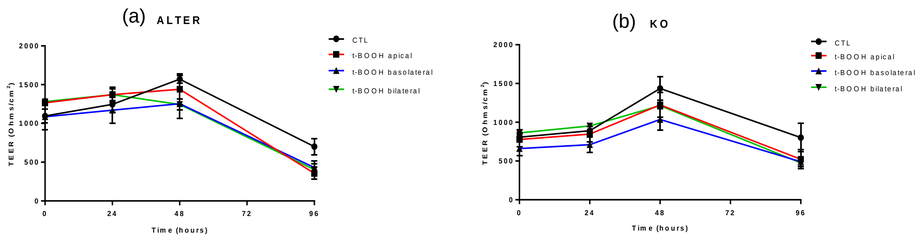

Figure 6. Changes in TEER measurements of ALTER (6a) and KO (6b) cells over 96 hours of exposure to t-BOOH apically, basolaterally and bilaterally (on both sides) compared to that of the Control (CTL).

Discusssion

- ASL pH was increased in response to t-BOOH of higher concentration.
- The results from pHi experiment suggested that the change in ASL pH in response to t-BOOH (1mM) was caused by a mechanism involving one or more of the Cl-/HCO_3- channels or transportes on the apical side.
- Decreased pHi in response to t-BOOH under high Cl- condition suggested t-BOOH activated HCO_3- secretion on apical side .
- Before exposure to t-BOOH, 0Cl- condition on the apical side did not cause a significant change in pHi, suggesting that the Cl-/HCO_3- exchangers were mostly inactivated then. When exposed to t-BOOH, 0Cl- caused a much larger increase in pHi, suggesting that t-BOOH has triggered a mechanism which activates the exchanger and caused it to reverse so that HCO_3- was transported into the cell.
- The small difference between changes in pH in the two cell lines suggested that these changes might not be caused by a mechanism involving CFTR channel.

Conclusion

- **t-BOOH of high concentration can elevate ASL pH** by increasing HCO_3- secretion from airway epithelial cells. However, the underlying **mechanism might not be CFTR-dependent**. In CF, this might help improve lung pathophysiology because it could be a defence mechanism to relieve the ASL acidity caused by defective anion secretion.
- TEER measurements showed that **t-BOOH can accelerate the reduction of epithelial integrity**. Therefore, it can weaken physical defence mechanism of the airway epithelium.
- Further studies need to be carried out in order to show that these results are reproducible on different cell lines and different ROS such as H_2O_2; also to confirm the underlying mechanism.

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Reference

[1] Berkebile AR, McCray PB, Jr. Effects of airway surface liquid pH on host defense in cystic fibrosis. The international journal of biochemistry & cell biology. 2014 Jul; 52:124-9