Distinct amino acids help to rectify dysfunctional epithelial chloride and sodium transport in primary human bronchial epithelial cells with CFTRΔF508 mutation

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BACKGROUND

Cystic fibrosis (CF) is a hereditary recessive disease caused by reduction or lack of CFTR synthesis, protein misfolding and/or channel dysfunction resulting in decreased chloride secretion, increased sodium absorption (ENaC), and impaired fluid homeostasis in airway, intestinal and pancreas epithelial cells. Recent advances in CF therapy using small molecules that selectively activate CFTR activity or correct protein misfolding have shown limited success in the CFTRΔF508 mutation. We have identified select amino acids (AA) combinations, that can stimulate chloride secretion and reduce ENaC activity by increasing apical chloride channel activity, and decreasing sodium absorption on the apical membrane.

MATERIALS AND METHODS

Cell model: Fully differentiated primary normal human bronchial epithelial cells (HBEC), and homozygous CFTRΔF508 HBEC (CF cells) were cultured on snapwells at an air-liquid interface for 28 to 40 days.

Using chamber: Transepithelial short-circuit current (Isc), resistance (R), and unidirectional (Iun) & Iun net flows (Jn) of 22Na and 22Cl were measured in normal HBECs and CF cells while bathing in vehicle, or CF-SAA-3 and CF-4AA-3. Chloride secretion was stimulated with forskolin (FSK, 10μM apical and basolateral) and the potentiatior GLPG1837 (3μM apical), and benzamil (6μM apical) and bumetanide (20μM apical and basolateral) were used to block ENaC and NKCC.

Statistic: Statistical differences between vehicle and AA formulations were calculated using analysis of variance (OriginPlus 2016). P < 0.05 was considered statistically significant.

RESULTS

Fig. 1. Basal Chloride Flux. Non-stimulated CF cells bathed in vehicle do not secrete chloride. However, chloride secretion was significantly increased in CF cells bathed in CF-SAA-3 (P < 0.05; n = 7).

Fig. 2. Basal Sodium Flux. Non-stimulated CF cells bathed in vehicle and CF-SAA-3 have a significantly higher sodium absorption compared to non-stimulated normal HBECs (P < 0.001; n = 6).

Fig. 3. Anion Currents and Stimulated Chloride Flux. A: The benzamil insensitive current (anion current) of CF cells bathed in vehicle was significantly lower compared to normal HBECs, but CF-SAA-3 could increase the anion current by a factor of > 10 (n = 4). B: The bumetanide sensitive current (chloride current) was significantly higher in CF cells bathed in CF-SAA-3 compared to vehicle (n = 4), but it did not reach the value of normal HBECs. C,D, E, F: CF cells bathed in CF-SAA-3 had a significantly higher anion peak current (E) and total chloride secretion (F), and stimulation with FSK and GLPG1837 did not contribute to the increased values in the presence of AA (C, D).

Fig. 4. ENaC Activity and Blocked Sodium Flux. A: CF cells bathed in vehicle or CF-SAA-3 had a significantly higher ENaC activity (benzamil sensitive current) compared to normal HBECs, but the ENaC activity was slightly lower in CF cells bathed in CF-SAA-3 compared to vehicle (n = 4). B: Similar, sodium absorption was significantly increased in CF cells with a moderate lower sodium absorption in CF cells bathed in CF-SAA-3 (n = 7).

Fig. 5. Effect of another AA formulation on ENaC activity in CF cells. CF cells bathed in CF-4AA-3 could further decrease the ENaC activity compared to vehicle and CF-SAA-3 (n = 4).

Conclusion

Select AA combinations can improve dysfunctional chloride and sodium channel activity in CFTRΔF508 HBEC by correcting and/or modifying plasma membrane channel function. These formulations could successfully complement existing standard of care in patients with the CFTRΔF508 mutation.