Assessment and pharmacological correction of abnormalities in bicarbonate (HCO3-) and mucus transport in intestinal biopsies and organoids of CF patients

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Background. Most physiological assays used for CF diagnosis and as a biomarker in clinical trials measure CFTR-dependent chloride but not bicarbonate transport. Recent studies however reveal that defective bicarbonate rather than chloride transport is the primary cause of impaired mucociliary clearance in the airways and luminal obstruction in the GI tract in CF.

Hypothesis and objectives. Our major aims were (i) development of new protocols to measure bicarbonate transport in rectal biopsies and intestinal organoids from healthy controls and CF patients; (ii) ex vivo testing of CFTR correctors and potentiators for their ability to restore bicarbonate secretion in these models; (iii) validating the concept that CFTR functional variants associated with pancreatitis and sinusitis but not CF have a specific bicarbonate permeation defect.

Methods. Chloride and bicarbonate transport in non-CF, CF patients (F508del) and non-CF pancreatitis patients (D1152H) were compared at the level of rectal biopsies and 3D and 2D intestinal organoids using a variety of techniques, including current measurements in Ussing chambers and organoid swelling assays. Moreover, we studied the ability of specific enzymes (WNK/SPAK kinases) to switch CFTR from a chloride into a bicarbonate conductive state.

Results. The bicarbonate secretion was 4-6 fold lower relative to chloride secretion in undifferentiated organoid monolayers from non-CF, and absent in F508del organoids. The corrector VX-809 and potentiator VX-770 rescued bicarbonate secretion in F508del organoids more efficient than chloride secretion (3-fold difference). Remarkably, bicarbonate and chloride secretion were of comparable magnitude in non-CF rectal biopsies, suggesting the operation of a bicarbonate importer in native colon that is low or absent in undifferentiated organoids. Calcium induced bicarbonate secretion was reduced much stronger than chloride secretion in biopsies from D1152H patients. The WNK-SPAK pathway was operative in non-CF organoids and stimulated by low intracellular chloride conditions. Experiments measuring the effect of D1152 and other variants before and after organoids differentiation are in progress.

Spin-off for research & clinical purposes. The protocols developed may facilitate future preclinical testing of novel CFTR repair molecules for their ability to restore bicarbonate transport in organoids from individual CF patients ("personalized medicine").

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