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Impaired distal colonic pH in adults with cystic fibrosis

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ABSTRACT

Previous wireless motility capsule (WMC) studies demonstrated decreased small intestinal pH in people with CF (PwCF) however the data is lacking on the colonic pH profile. We re-analyzed previously published WMC data to determine colonic pH/bicarbonate concentration and single cell RNA sequencing (sc-RNAseq) to examine the normal expression of acid-base transporters in the colon/rectum.CF patients showed significantly lower pH and bicarbonate concentration values, particularly in the distal rectosigmoid region. There was no difference in colonic motility parameters between CF and non-CF subjects. SLC26A3 is highly expressed bicarbonate transporter in the colon and rectum, more so than CFTR. While dysmotility can alter intraluminal pH, observed changes likely originate from alterations in intestinal ion transport rather than colonic dysmotility. SLC26A3 is abundantly expressed in the human colon and rectum and may be a therapeutic target for restoration of bicarbonate transport. These findings may help better understand the gastrointestinal symptoms in PwCF

1. Introduction

Gastrointestinal pH is crucial for physiological gut functioning. Variations in pH along the gastrointestinal tract in health are designed for nutrient digestion and absorption and provide innate defense against ingested microorganisms [1]. Small intestinal pH is influenced by the expression and function of mucosal acid transporters (e.g., *SLC9A3*) and base transporters (e.g., *CFTR, SLC26A3, SLC26A6*) along with alkaline-rich pancreatic and biliary secretions [2,3]. Colon pH is thought to be regulated by many of the same acid-base transporters but may also be influenced by colonic bacterial fermentation, production of carbon dioxide, and short-chain fatty acids that are transported in a proton-coupled manner via *SLC16A1* [4,5].

Small intestinal pH is decreased in cystic fibrosis (CF) due, at least in part, to decreased CFTR-mediated bicarbonate secretion [6]. It remains unknown if colonic pH is altered in CF. Given the observed base transport disturbances and dysmotility in other regions of the gastrointestinal tract in CF, we hypothesized that the colon may also display altered intestinal pH and this may contribute to chronic abdominal pain, distal intestinal obstruction syndrome, bloating, and chronic constipation experienced by many persons with CF.

To address this hypothesis, we re-analyzed prior wireless motility capsule (WMC) studies in CF and non-CF subjects [7] to determine pH, estimated bicarbonate concentrations, and motility profile of the distal colon. We also re-analyzed prior single-cell RNA sequencing (scRNA-seq) studies of human non-CF colon and rectum to characterize acid-base transporters of the colon and rectum. This combination of WMC and scRNA-seq studies provide us new insights into colonic pathophysiology in CF.

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2. Methods

2.1. Human subjects

We conducted an expanded analysis of WMC data previously collected from individuals with CF and non-CF matched control subjects [7]. All data was de-identified and Institutional Review Board exempt. Data was obtained from 10 CF subjects, ≥18 years-old with exocrine pancreatic insufficiency, and were at baseline health with no hospitalization within the 2 months preceding the WMC. No subjects were on CFTR modulator therapies, gastric acid inhibitors, or inhaled antibiotics during the study period. Each CF subject underwent two independent WMC studies, generally 8 weeks apart. Healthy control subjects were matched by age ±3 years, sex, and body mass index ±2 kg/m^2.

2.2. pH measurements

The WMC (Medtronic Corporation, Minneapolis MN) possesses sensors to measure pH, pressure and temperature for approximately 72 h, as previously described [7]. All subjects passed the WMC within 72 h. Data was extracted using GIMS software (Gastrointestinal Motility Software [GIMS^{TM}] Medtronic Corporation, Minneapolis MN). Anal exit was defined as an acute temperature drop and permanent loss of signal on the receiver. We measured pH and pressure changes from the distal colon, divided into 4 segments, each 30-min intervals before the anal exit of the WMC. Mean pH values over 30-min increments were calculated. For comparison, gastric measurements were taken prior to gastric emptying, defined as an abrupt rise in the pH by 3 or more units above the stomach pH.

2.3. Calculation of bicarbonate concentration

To estimate the colonic bicarbonate concentration ([HCO_3^-]), we utilized the Henderson-Hasselbach equation, which considers the proton acceptor (HCO_3^-) and the proton donor (CO_2) to determine the pH, $[HCO_3^-] = 0.03 \times pCO_2 \times 10^{(pH - 6.1)}$. Due to the absence of human colonic pCO_2 data, previously published porcine sigmoid colon pCO_2 values were used [8].

2.4. Single-Cell rna sequencing analysis

The R toolkit Seurat [9] was used to perform quality control and analysis of human colonic and rectal single-cell RNA sequencing (scRNA-seq) data from a previously published study on non-CF subjects [10]. In brief, we embedded the cells through a K-nearest neighbor graph and reduced the data by principal component analysis. We visualized single-cell gene expressions per cell type as annotated by Wang *et al.* [10] with Violin plots in Seurat.

2.5. Statistics

Means and standard deviations were computed for all measurements. Non-parametric Mann-Whitney *t*-test or analysis of variance was used to evaluate the differences between CF and control data using GraphPad Prism 9.4 (San Diego, CA). Significance was set to $P<0.05$.

3. Results

3.1. Colonic pH and bicarbonate concentration

Colonic pH from WMC measurements in control subjects showed similar median pH values of 7.46, 7.60, 7.53 at the proximal time points [2, 1:30, and 1 h prior to exit, respectively), with the most distal measurements having a generally higher pH of 7.88, although this was not significant ($P = 0.280$, Fig. 1A). In comparison, median colonic pH measurements in CF subjects were similar across all time points at: 6.58, 6.54, 6.58, 6.53 (proximal to distal) and were significantly less than control subjects, most notably in the most distal measurements (Fig. 1A). Calculation of [HCO_3^-] showed similar trends in control (median proximal to distal [HCO_3^-] = 55.1, 63.8, 46.2, and 121.9 mM, respectively) and CF subjects (median proximal to distal [HCO_3^-] = 6.2, 5.6, 6.1, and 5.5 mM, respectively) (Fig. 1B).

3.2. Colonic motility

To determine if colonic dysmotility may contribute to decreased colonic pH in CF, we examined the contractions and motility parameters at the same timepoints that pH were measured. As seen in Table 1, there were no significant differences in the number of contractions, contractions per minute, the Camilleri or Ouyang motility indices, or the area under the pressure curve between control and CF subjects. To verify WMC's ability to identify dysmotility, we examined stomach motility, given the high prevalence of gastroparesis in CF [11]. As seen in Table 1, we found low stomach motility indices in CF and significant differences compared to control subjects.

3.3. Expression of colonic acid-base transporters

With dysmotility likely not playing a significant role in decreased colonic and rectal pH in CF subjects, we next examined whether the decreased colonic pH and [HCO_3^-] observed in CF might be due primarily to decreased CFTR expression or potentially to other acid-base transporters. ScRNA-seq of 4472 colonic cells and 3898 rectal cells from non-CF subjects ($n = 4$) showed colonic expression profiles of acid-base transporters in enterocytes (greatest to least) as: *SLC26A3*>>*CFTR*≈*SLC16A1*>*SLC26A6*. For rectum: *SLC26A3*>>*SLC16A1*≈*SLC26A6*>*CFTR*. Acid transporter *SLC9A3* was not detected in either colon or rectum (Fig. 2).

4. Discussion

Gastrointestinal pH is physiologically variable along the small and large intestine. In mouse and human studies, including with the WMC, loss of CFTR function in CF decreases proximal small intestine bicarbonate secretion and pH [7,12,13]. In health, intestinal pH decreases in the cecum and then gradually increases distally towards the rectum. Decreased colonic pH has been noted in irritable bowel syndrome [14] and inflammatory bowel disease [15], however, the colonic pH profile in CF has not been investigated.

In our current study we found that WMC-measured distal colonic pH was markedly lower in CF subjects than healthy control subjects. Colonic pH may be influenced by mucosal bicarbonate and proton transport, lactate production, bacterial fermentation of carbohydrates, absorption of short chain fatty acids (coupled with proton transport), and intestinal transit. Due to the retrospective nature of our analysis, we did not have data on the presence or absence of subclinical intestinal inflammation or the colonic microbiome so we cannot ascertain to what degree, if any, these may have contributed to our findings. However, with the WMC we were able to evaluate colonic motility through contraction measurements. Our findings that distal colon and rectal contractions in CF subjects were similar to healthy control subjects argues against impaired colonic motility being responsible for our findings of decreased intraluminal pH. While manometry is the gold-standard for motility, this data is lacking in CF. In non-CF subjects, WMC detected 86% of contractility events (compared to simultaneous manometry) with a negative predictive value of 99.9%

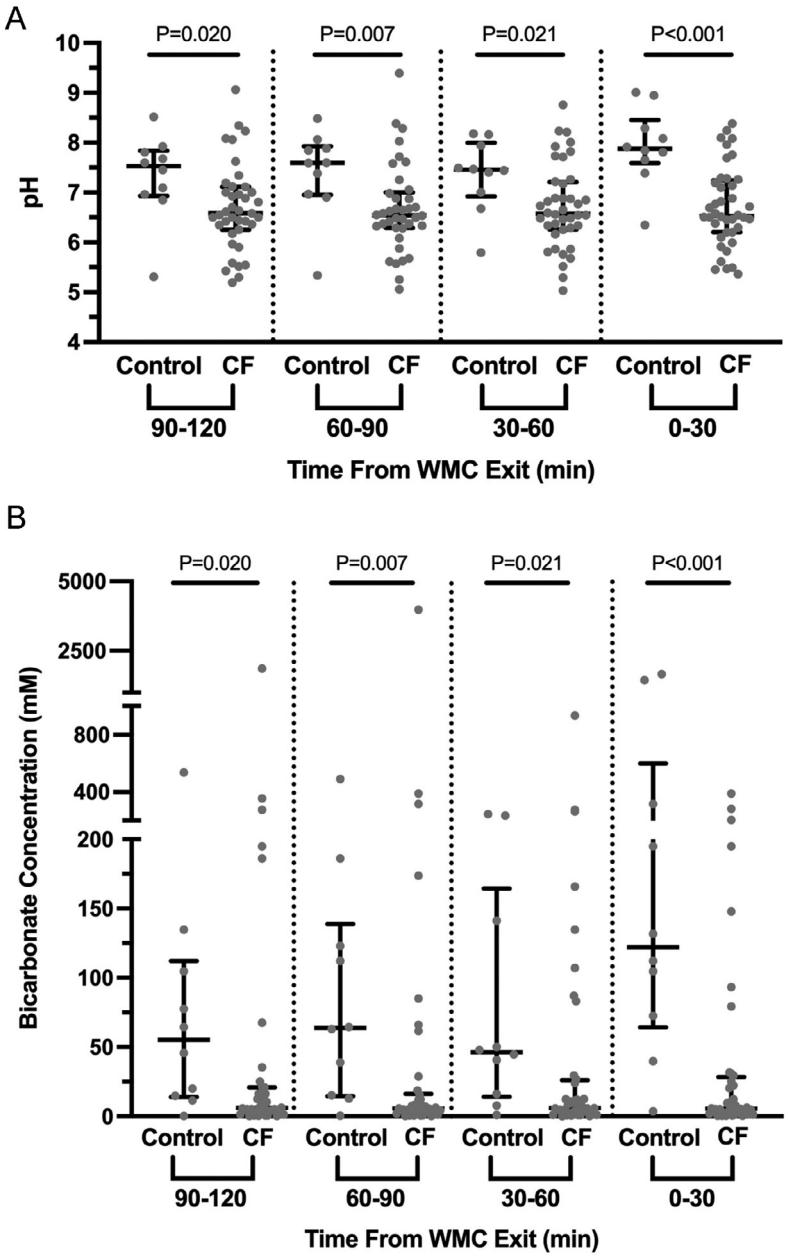

Fig. 1. Decreased distal colonic pH and bicarbonate concentration in CF compared to controls based on WMC. A. The average pH recordings over 30 min for each interval was calculated and is shown for each of the 4 intervals, with proximal indicating 2 h prior to exit and distal the last 30 min prior to exit. Dots indicate results from each subject. Median with 95% confidence intervals is indicated by black bars. Significance was determined by non-parametric Mann-Whitney *t*-test. B. pH recordings from Fig. 1A were used to calculate bicarbonate concentrations and are presented in the same manner as Fig. 1A.

[16,17]. Therefore, WMC may serve as a good surrogate marker for noninvasive measure of GI motility in CF. Additionally, magnetic resonance imaging-based colonic transit measurements in CF identified proximal, but not distal, colonic dysmotility, similar to our findings [18].

Rectal biopsies from CF patients have been used to identify loss of CFTR mediated transport by short-circuit and organoid swelling [19–21]. However, there is no data on intraluminal bicarbonate concentration, bicarbonate transport processes, or even the expression profile of acid-base transporters in the distal colon and/or rectum. *Re*-analyzing existing scRNA-seq data we identified that DRA (*SLC26A3*) is highly expressed bicarbonate transporter in the colon and rectum, even more so than *CFTR*. In human proximal colonoids, Tse et al. found that DRA activity was influenced by the expression of CFTR [22]. It is unclear if the profound decrease in rectal pH and bicarbonate concentration we observed was due to loss of CFTR-mediated bicarbonate secretion alone or to a combination of CFTR- and DRA-mediated bicarbonate transport. Further research into how loss of CFTR affects other acid-base transporters in the colon will be important.

Our current study has several limitations. The WMC has only one pressure sensor, it cannot measure contraction related propagation between two points. Hence, we were not able to define details of high amplitude propagating contractions (HAPC) which are considered hallmark of normal neuromuscular integrity of the colon. WMC also does not provide information on exact location. We attempted to overcome this by using predefined windows from anal exit. Bicarbonate concentration values are estimates rather

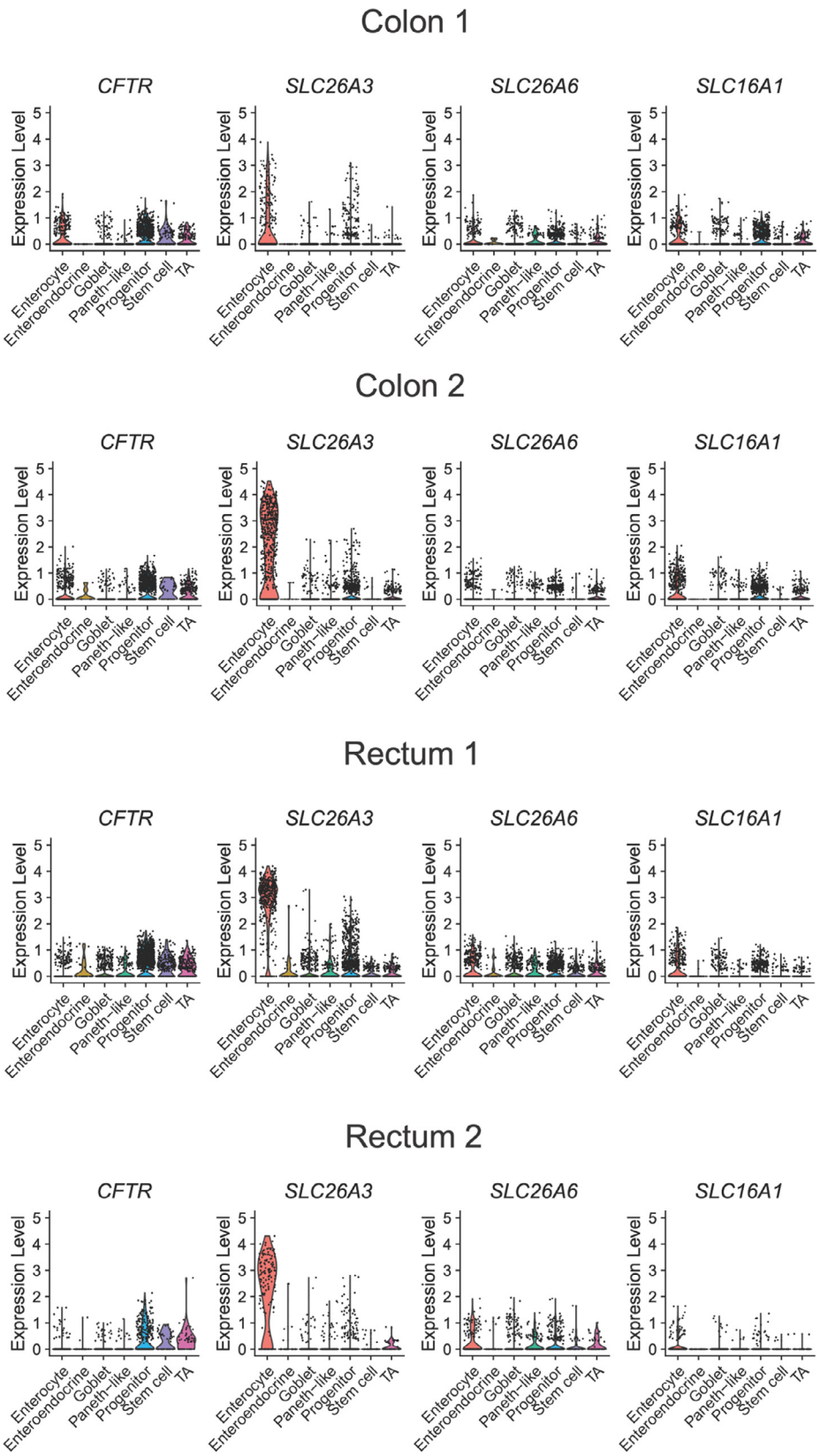

Fig. 2. Single cell RNA sequencing of human colon and rectum. Prior scRNA-seq data from Wang et al. (10) was used to perform analysis on the mRNA expression of *CFTR*, *SLC26A3* (down-regulated in adenoma, DRA), *SLC26A6* (putative anion transporter-1, PAT-1), and *SLC16A1* (monocarocylate transporter 1, MCT1) from 4472 colonic cells (A) and 3898 rectal cells (B) from four non-CF subjects. Violin plots represent expression relative to all cells of that type, with each dot representing the expression of individual cells within each cell type.

Table 1
No evidence of distal colonic dysmotility in CF with WMC, despite decreased gastric motility profiles.

	Controls (n = 10)	CF (n = 39)	P Value
Distal Colon			
Number of Contractions	86.7 (114.0)	111 (121.8)	0.5619
Contractions Per Minute	0.09 (0.10)	0.13 (0.14)	0.2867
Motility Index - Camilleri	12.3 (2.2)	12.64 (2.59)	0.6785
Motility Index - Ouyang	24.83 (27.88)	28.97 (48.09)	0.7270
Area Under Pressure Curve	22,068 (29,561.2)	26,867.3 (48,438.4)	0.6964
Stomach			
Number of Contractions	184.7 (137.8)	82.6 (59.17)	0.0011
Contractions Per Minute	0.86 (0.72)	0.4 (0.26)	0.0019
Motility Index - Camilleri	13.86 (1.33)	12.28 (1.22)	0.0026
Motility Index - Ouyang	71.87 (63.09)	33.31 (14.04)	0.0009
Area Under Pressure Curve	14,213.5 (11,064.2)	6684.5 (2618.5)	0.0003

than actual values since intraluminal pCO_2 measurements were not obtained at the time of WMC. ScRNA-seq data was only available from non-CF patients and mRNA and protein membrane expression may be discordant. Finally, our scRNA-seq data involved analysis only of non-CF subjects. We did not compare transcriptomic analysis of acid-base transporter expression in subjects with CF, although this would be an important next step to deepen our understanding how loss of CFTR expression and/or function impacts intestinal pH. Despite these limitations, our study uses a combination of clinical and laboratory techniques to bring new insight into colonic pH in CF. We hope that the data generated in this pilot study will help spur further research in the clinical utility of measuring colorectal pH in CF, pathogenesis of CF colonic disease, and potential new targets to ameliorate GI complications in CF.

Authors' contribution statement

Dhiren Patel: Substantial contributions to the conception or design of the work, Acquisition, analysis, or interpretation of data for the work, Drafting the work or revising it critically for important intellectual content, Final approval of the version to be published, Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work.

Stacy Mathews: Substantial contributions to the conception or design of the work, Acquisition, analysis, or interpretation of data for the work, Drafting the work or revising it critically for important intellectual content, Final approval of the version to be published, Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work.

Vincent van Unen: Acquisition, analysis, or interpretation of data for the work, Drafting the work or revising it critically for important intellectual content, Final approval of the version to be published, Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work.

Joshua E. Chan: Acquisition, analysis, or interpretation of data for the work, Drafting the work or revising it critically for important intellectual content, Final approval of the version to be published, Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work.

Noor Al-Hammadi: Acquisition, analysis, or interpretation of data for the work, Drafting the work or revising it critically for important intellectual content, Final approval of the version to be published, Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work.

Drucy Borowitz: Substantial contributions to the conception or design of the work, Acquisition, analysis, or interpretation of data for the work, Drafting the work or revising it critically for important intellectual content, Final approval of the version to be published, Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work.

Daniel Gelfond: Substantial contributions to the conception or design of the work, Acquisition, analysis, or interpretation of data for the work, Drafting the work or revising it critically for important intellectual content, Final approval of the version to be published, Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work.

Zachary Sellers: Substantial contributions to the conception or design of the work, Acquisition, analysis, or interpretation of data for the work, Drafting the work or revising it critically for important intellectual content, Final approval of the version to be published, Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work.

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Declaration of Competing Interest

All authors report no conflict of interest related to this study.

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