Title

Safety, Tolerability, and Effects of Sodium Bicarbonate Inhalation in Cystic Fibrosis

Journal

Clinical Drug Investigation, 40(2)

ISSN

1173-2563

Authors

Gomez, Carla Cristina Souza

Parazzi, Paloma Lopes Francisco

Clinckspoor, Karl Jan

et al.

Publication Date

2020-02-01

DOI

10.1007/s40261-019-00861-x

Peer reviewed

Clinical Drug Investigation
https://doi.org/10.1007/s40261-019-00861-x

ORIGINAL RESEARCH ARTICLE

Safety, Tolerability, and Effects of Sodium Bicarbonate Inhalation in Cystic Fibrosis

Carla Cristina Souza Gomez[1] · Paloma Lopes Francisco Parazzi[1] · Karl Jan Clinckspoor[2] · Renan Marrichi Mauch[1] · Francisco Benedito Teixeira Pessine[2] · Carlos Emilio Levy[3] · Andressa Oliveira Peixoto[1] · Maria Ângela Gonçalves Oliveira Ribeiro[1] · Antônio Fernando Ribeiro[1] · Douglas Conrad[4] · Paul Marquis Quinton[5] · Fernando Augusto Lima Marson[1,6,7] · José Dirceu Ribeiro[1]

Abstract

Background Among the many consequences of loss of CFTR protein function, a significant reduction of the secretion of bicarbonate (HCO_3^-) in cystic fibrosis (CF) is a major pathogenic feature. Loss of HCO_3^- leads to abnormally low pH and impaired mucus clearance in airways and other exocrine organs, which suggests that $NaHCO_3$ inhalation may be a low-cost, easily accessible therapy for CF.

Objective To evaluate the safety, tolerability, and effects of inhaled aerosols of $NaHCO_3$ solutions (4.2% and 8.4%).

Methods An experimental, prospective, open-label, pilot, clinical study was conducted with 12 CF volunteer participants over 18 years of age with bronchiectasis and pulmonary functions classified as mildly to severely depressed. Sputum rheology, pH, and microbiology were examined as well as spirometry, exercise performance, quality-of-life assessments, dyspnea, blood count, and venous blood gas levels.

Results Sputum pH increased immediately after inhalation of $NaHCO_3$ at each clinical visit and was inversely correlated with rheology when all parameters were evaluated: [G' (elasticity of the mucus) = − 0.241; G'' (viscosity of the mucus) = − 0.287; G^* (viscoelasticity of the mucus) = − 0.275]. G^* and G' were slightly correlated with peak flow, forced expiratory volume in 1 s (FEV_1), and quality of life; G'' was correlated with quality of life; sputum pH was correlated with oxygen consumption (VO_2) and vitality score in quality of life. No changes were observed in blood count, venous blood gas, respiratory rate, heart rate, peripheral oxygen saturation of hemoglobin (SpO_2), body temperature, or incidence of dyspnea. No adverse events associated with the study were observed.

Conclusion Nebulized $NaHCO_3$ inhalation appears to be a safe and well tolerated potential therapeutic agent in the management of CF. Nebulized $NaHCO_3$ inhalation temporarily elevates airway liquid pH and reduces sputum viscosity and viscoelasticity.

Key Points

Inhalation of aerosolized $NaHCO_3$ was safe and well tolerated by CF patients.

There were no significant adverse events associated with the intervention.

The apparent benefits of inhaled $NaHCO_3$ most likely are due to its role in raising the pH of the airway surface liquid along with concomitant effects on sputum rheology and other parameters.

Fernando Augusto Lima Marson and José Dirceu Ribeiro contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s40261-019-00861-x) contains supplementary material, which is available to authorized users.

✉ Carla Cristina Souza Gomez
carlacg.gomez@gmail.com

✉ Fernando Augusto Lima Marson
fernandolimamarson@hotmail.com

✉ José Dirceu Ribeiro
jdirceuribeiro@gmail.com

Extended author information available on the last page of the article

Published online: 13 November 2019

1 Introduction

In recent years knowledge regarding the pathophysiology of pulmonary disease in cystic fibrosis (CF; OMIM: #219700) has expanded significantly from studies on ion transport by epithelial anion channels in affected tissues [1, 2]. The early literature reported that viscous, thick mucus with high concentrations of solids that obstructed the airways in CF was mainly caused by dehydration of airway surface liquid (ASL) due to dysfunction in chloride transport by the CFTR (cystic fibrosis transmembrane conductance regulator) protein and presumably concomitant sodium/water hyperabsorption from airway luminal fluid [3], which was thought to provide a hypoxic environment favoring bacterial growth [4]. However, recent studies found that defects in the CFTR protein also reduced transport of bicarbonate (HCO_3^-), which is essential for developing and maintaining the physiological properties of mucins [5, 6]. Mucins create a gel matrix to lubricate epithelial surfaces and act as a barrier against pathogens [7]. Reduction or absence of HCO_3^- prevents normal expansion and maturation of mucins since HCO_3^- must be physiologically secreted with mucin exocytosis to capture calcium (Ca^{2+}) and buffer free hydrogen (H^+) [8–10]. Reduced HCO_3^- may also favor growth of bacteria, increased viscous mucus production and biofilm formation, and depressed immune responses consequent to reduced pH of the ASL [11–13].

In CF, besides the direct effects of low levels of HCO_3^- on the viscoelastic properties of mucus, other factors such as increased elastase, DNA nets, and oxygen free radicals from neutrophil apoptosis are released in the airways [7] as a consequence of inflammation and infection, which furthers deterioration of lung function, the primary cause of morbidity and mortality in CF [14].

Only relatively recently were the abnormalities of mucociliary transport in CF suggested to be caused by a HCO_3^- deficiency in ASL [8]. In vitro, $NaHCO_3$ was shown to change the rheological properties of sputum [15]. Thus, in CF, three factors seem to justify the therapeutic use of inhaled HCO_3^- aerosol: (1) HCO_3^- is critical in the extracellular processing of mucus to attain physiologically rheological properties [5, 8, 10, 11, 16]; (2) increased ASL pH decreases bacterial proliferation [13]; (3) hyperosmotic solutions of $NaHCO_3$ should help mobilize and remove airway surface debris [17, 18].

In this context, the objective of this study was to evaluate the safety and tolerability of inhaling aerosolized $NaHCO_3$ as the primary outcome in light of its potential effect on clinical and laboratory markers in CF as secondary outcomes.

2 Method

This study was a phase I longitudinal non-randomized, unblinded clinical study of inhaled aerosolized $NaHCO_3$ with dose escalation in volunteer CF participants performed at a single site. The study determined safety and tolerability and assessed clinical and laboratory markers following a modified PICOT strategy:

(P) Population: CF patients over 10 years of age with two sweat tests with a sweat chloride concentration ≥ 60 meq/L and/or two pathogenic variants in the *CFTR* gene (OMIM: #602421). Twelve of 19 volunteers with CF (nine females) over 18 years of age were included and followed upto completion of the protocol. In addition, nine pancreatic insufficient (PI) patients were included. All subjects were diagnosed with bronchiectasis from computer-assisted tomography (CAT) images of the thorax, with pulmonary functions, including forced expiratory volume in 1 s (FEV_1) reductions, assessed as mild to severe. Participants included in this study were clinically stable, on continuous use of oral and/or inhaled antibiotics, and were able to expectorate spontaneously (Table 1).

(I) Intervention: Serial doses of 5 mL of 4.2% or 8.4% $NaHCO_3$ dissolved in distilled water were inhaled as an aerosol mist.

(C) Control: Temporal analysis in the same individual relative to $NaHCO_3$ administration. There was no placebo, active drug, or healthy subjects that served as a control arm.

(O) Outcomes: The primary outcome was safety and tolerability of inhaling aerosolized $NaHCO_3$. In addition, the following parameters were evaluated as secondary outcomes: physical/chemical characteristics of sputum, pulmonary function, Harvard step-test performance, sputum microbiology, serum electrolytes, blood count, and questionnaire scores for assessing quality of life and dyspnea.

(T) Time: Ten weeks. Study parameters were assessed and recorded after weekly visits during the 10-week study protocol.

The project (#12398956) was approved by the research ethics committee of the Faculdade de Ciências Médicas, Universidade Estadual de Campinas. Informed consent was obtained from each participant.

2.1 Intervention Model

In the study, each participant underwent a screening visit (1) to establish pre-intervention values of parameters to

Table 1 Clinical and demographic characteristics of participants with cystic fibrosis for inhaled sodium bicarbonate

Patient	Age (years)	Sex	*CFTR* genotype	*CFTR* class	PI	FEV_1	Discontinued	Reason to discontinue
P1	19	Female	F508del/G542X	II/I	No	Mild	Yes	Insufficient sputum
P2	31	Female	A561E/A561E	II/II	Yes	Moderate	No	
P3	29	Female	F508del/–	II/–	No	Severe	No	
P4	18	Male	F508del/G542X	II/I	Yes	Moderate	Yes	Pulmonary exacerbation
P5	17	Male	F508del/–	II/–	No	Mild	No	
P6	24	Male	F508del/G542X	II/I	Yes	Moderate	No	
P7	26	Male	2183AA>G/N1303 K	I/II	Yes	Severe	Yes	Did not complete study stages
P8	20	Female	F508del/F508del	II/II	Yes	Severe	No	
P9	16	Female	F508del/–	II/–	Yes	Severe	Yes	Use of oxygen
P10	19	Female	F508del/–	II/–	No	Mild	No	
P11	13	Male	F508del/–	II/–	Yes	Mild	Yes	The participant did not have time to participate in the study
P12	19	Male	F508del/F508del	II/II	Yes	Severe	No	
P13	21	Male	F508del/–	II/–	Yes	Mild	No	
P14	23	Female	F508del/G542X	II/I	Yes	Moderate	Yes	Pulmonary exacerbation
P15	28	Male	F508del/–	II/–	Yes	Severe	No	
P16	43	Male	F508del/F508del	II/II	Yes	Severe	Yes	The participant did not have time to participate in the study
P17	21	Male	F508del/F508del	II/II	Yes	Mild	No	
P18	26	Female	F508del/F508del	II/II	Yes	Severe	No	
P19	22	Male	F508del/F508del	II/II	Yes	Moderate	No	

PI pancreatic insufficiency—assessment of pancreatic status was evaluated measuring fecal elastase in stool or fecal fat in 72-h stool collections in the routine diagnostic tests. Pancreatic enzyme replacement therapy was continued for cystic fibrosis (CF) patients with PI [24]. Detailed information regarding the pathogenic variants in the *CFTR* gene is shown as: G542X-c.1624G>T, p.Gly542X, rs113993959; F508del-c.1521_1523delCTT, p.Phe508del, rs113993960; A561E-c.1682C>A, p.Ala561Glu, rs121909047; 2183AA>G-c.2051_2052delAAinsG, p.Lys684SerfsX38, rs121908799; N1303K-c.3909C>G, p.Asn1303Lys, rs80034486; FEV_1 forced expiratory volume in 1 s of the forced vital capacity (FVC); *CFTR* Cystic Fibrosis Transmembrane Conductance Regulator

The spirometry FEV_1 values were classified as mild (> 60%), moderate (41–59%), or severe (≤ 40%)

be assessed for effects during and post-intervention, and (2) to train for $NaHCO_3$ inhalation and receive a supply of 5-mL doses of 4.2% or 8.4% $NaHCO_3$ solution for home inhalation according to the following protocol, which was comprised of five treatment regimens in the following sequence: (1) 4.2% (once a day) for 2 weeks; (2) 4.2% (twice daily) for 2 weeks; (3) 8.4% (once a day) for 2 weeks; (4) 8.4% (twice daily) for 2 weeks; (5) no $NaHCO_3$ for 2 weeks (wash-out period). Each patient was evaluated clinically during the initial screening visit and during each visit after each dose regimen. CF participants did not use inhaled hypertonic saline during the intervention, but four patients continued the use of inhaled dornase alpha. Inhalation of hypertonic saline was avoided because it is used therapeutically to enhance sputum production. Even though the mechanisms of action of hypertonic saline and $NaHCO_3$ inhalation are not the same, the concomitant use of both drugs would inherently complicate the evaluation of the effects of $NaHCO_3$. No alteration or interruption of the participants' usual treatments occurred as a result of the use of $NaHCO_3$ inhalation during the intervention period. However, bicarbonate inhalation was scheduled to be the first inhalation treatment of the day, and the participants were advised to delay performing respiratory physiotherapy immediately after the bicarbonate inhalation in order to delay any effects on immediately clearing the drug and to avoid the impact of respiratory physiotherapy on clearance of airway secretions; both factors would complicate the interpretation of effects of $NaHCO_3$ inhalation.

The order of the aerosol solutions was not evaluated. Additionally, participants taking inhaled antibiotics at the beginning of the study were advised to continue their prescribed use so as to avoid introducing an extraneous variable affecting pulmonary function. Participants being treated with aerosolized antibiotics were asked to inhale mucolytics first.

2.2 Clinical and Laboratory Markers

2.2.1 Elasticity, Viscosity, and pH of Sputum

Sputum was collected during all visits at intervals of 30 min over a 180-min period after the inhalation treatment as well as before and after the spirometry tests and Harvard step tests. A minimum sputum specimen of 3 mL was collected in a 15-mL sterile tube and frozen in dry ice for storage and transport. Rheological analysis evaluated specimen: (1) elasticity – G' (stiffness); (2) viscosity – G'' (flow resistance); and (3) viscoelastic modulus – G^* [15, 19] (see Electronic Supplementary Material (ESM) 1). The rheology analysis was performed using a Haake RheoStress 1 rheometer (Thermo Scientific®, Waltham, MA, USA), according to the manufacturer's instructions. All rheology analyses were performed at the Department of Physics and Chemistry, Instituto de Química, Universidade Estadual de Campinas.

The pH was measured concomitantly with the rheology in a ST2100 pH meter (OHAUS®, Parsippany, NJ, USA) using a pH electrode (Digimed®, São Paulo, Brazil) made specifically for fluids containing protein, fat, and/or creams. Room temperature was maintained at 23 °C. Specimens were equilibrated with room air and temperature, but not controlled for CO_2 tension.

2.2.2 Microbiology of Sputum

A semi-quantitative analysis was made of bacteria in the sputum specimens collected at the initial screening visit, V0, and at the final visit, V5. The bacterial load was classified as (1) +: light; (2) ++: moderate; and (3) +++: heavy. All specimens were assayed for the presence of mucoid and non-mucoid *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

2.2.3 Spirometry

Spirometry was performed according to the American Thoracic Society and European Respiratory Society guidelines using a Koko® Spirometer (nSpire Health, Longmont, CO, USA) [20]. At least three well-defined and reproducible maximum flow-volume loops were obtained: (1) before, (2) after administering the bronchodilator Salbutamol ($C_{13}H_{21}NO_3$, 400 µg), and (3) after conducting the Harvard step-test exercise. The following parameters were measured: forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), and forced expiratory flow rate between 25 and 75% of the FVC ($FEF_{25–75\%}$). The best result of three sequential exhalation efforts was recorded.

2.2.4 Peak Flow

Participants used a peak-flow meter according to the guidelines of the American Lung Association [21]. The best of three measurements taken during the clinic visits and at home (twice daily) were recorded.

2.2.5 Harvard Step Test

The step height was set at 30 cm (kikos™, São Paulo, Brazil). The following parameters were recorded:

1. Fitness index (FI) calculated as [(100 × test duration in s)/(2 × sum of heart beats in the recovery period)], which was classified as: excellent (> 90), good (80–89), above average (65–79), low (55–64), or insufficient (< 55).
2. Maximum oxygen consumption calculated as (VO_2 max (mL/kg/min) calculated as 0.2 × (stepping rate) + [1.33 × 1.8 × (step height in meters) × (steps frequency/min)] + 3.5 (resting component).
3. Energy expenditure calculated as [VO_2max (mL/kg/min) × weight (kg)]/1000 × 5 kcal/L], which converts VO_2 max into expended energy (kcal/min).

2.2.6 Cystic Fibrosis (CF) Quality-of-Life Questionnaire (CFQoLQ) and San Diego Questionnaire (SDQ) for Dyspnea

Participants answered the standard questionnaire at all visits [22, 23].

2.2.7 Adverse Events

To assess the tolerability and possible side effects of inhaling aerosolized $NaHCO_3$ over time, participants completed a daily recall form (medications administered; visual analogue scale for dyspnea; cough activity; respiratory congestion, as well as other clinical indicators including pulmonary exacerbations, sinusitis, hemoptysis, malaise or lack of energy, weight loss, fever, absence from work/school.

At all visits, the following were measured: heart rate, respiratory rate, peripheral oxygen saturation of hemoglobin (SpO_2), weight, arterial blood pressure, and body temperature. In addition, an automatic blood cell count (red blood cell, white blood cells, and platelets) as well as venous blood gas levels were obtained at the initial visit, V0, and the final visit, V5.

2.2.8 Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences version 24.0 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Armonk, NY, USA). The statistical design is shown in ESM 1.

3 Results

Initially, 19 volunteer participants with CF, who were not using aerosolized hypertonic saline inhalation therapy, were enrolled. Seven of these 19 volunteers were excluded from the study due to complicating conditions: (1) insufficient sputum production to perform the rheology measures ($n=1$), (2) poor adherence to the protocol ($n=1$) (participant used less than 80% of the drug dose); (3) oxygen therapy required prior to beginning the intervention ($n=1$) [oxygen therapy was prescribed by a physician at the screening visit, V0, and the trial investigator was informed], (4) insufficient time for weekly visits ($n=2$), (5) pulmonary exacerbations at the onset of the study ($n=2$) [during the clinical evaluation two physicians concluded the incidents observed as exacerbations were ongoing with prior bacterial colonization. Even though it seems unlikely, we cannot discard the possibility that the intervention was a contributing factor, and we note them as possible adverse events. Importantly, the exacerbation appeared during the first period of inhalation and was noted as a clinical finding during the first visit, V1. Consequently, both patients were excluded from the study and no data were collected from these patients]. All 12 participants who completed the protocol were more than 18 years of age and were diagnosed with bronchiectasis; five were female; nine were pancreatic insufficient. Their spirometry FEV_1 values were classified as mild (> 60%; $n=6$), moderate (41–59%; $n=5$), or severe (≤ 40%; $n=8$) (Table 1). Four patients continued inhaling aerosolized dornase alpha as therapy during the protocol.

To assess adherence, the participants responded to a questionnaire concerning their routine healthcare. An additional questionnaire with subjective questions regarding fatigue, daily sputum characteristics, and symptoms associated with dyspnea were scored. Incomplete questionnaires delivered during the visits were interpreted as insufficient interest to participate in the study. Concomitantly, the drug supplied to the participant was dated with the correct day for use, and the participant was instructed to return all empty and full vials to the study personnel on each subsequent visit.

Pertaining directly to the safety and tolerability of inhaling aerosolized $NaHCO_3$, no changes were observed in: complete blood count, venous blood gas, heart rate, respiratory rate, SpO_2, body temperature, or the dyspnea questionnaire (data not shown; $p \geq 0.05$). Participants reported no serious adverse events. One participant complained of a burning sensation on the lips, which was resolved by ingesting water during inhalation, and recommending exhaling outside the mouthpiece. Two participants reported cough during the first inhalation, which did not re-occur subsequently.

3.1 Rheology, Spirometry, Microbiology

Comparison of the rheology values (G', G'', and G^*) between visits and during the visits did not show significant differences ($p > 0.05$) (ESM 2). Spirometry and microbiology results did not differ during the intervention (Table 4; ESM 3).

3.2 pH

After $NaHCO_3$ was inhaled, the pH of the first specimen of sputum taken each day was the highest of all subsequent specimens and showed statistically higher values in visits V3 and V4 than at the screening visit (V0) (8.4%, $p=0.004$ and 0.001) (Table 2; Fig. 1). Furthermore, at all visits after inhalation of $NaHCO_3$ aerosol, the mean pH of samples collected between time zero and 180 min decreased with time to the pH of the screening sample at V0 ($p < 0.05$) (Table 2). Despite being an exploratory and underpowered analysis, an inverse correlation was observed between pH and rheology markers when all data were considered ($G' = -0.241$; $G'' = -0.287$; $G^* = -0.275$) (Table 5) and the mean value obtained at each visit ($G' = -0.312$; $G'' = -0.384$; $G^* = -0.336$) ($p < 0.001$) (ESM 4). Oxygen consumption ($p=0.025$), energy expenditure ($p=0.024$), quality-of-life markers [treatment burden ($p=0.010$); health perceptions ($p=0.011$) and total score ($p=0.009$)] showed improvement during the intervention period (Tables 2, 3). Other significant correlations were obtained with markers for: (1) G^* versus peak flow, FEV_1, quality of life (vitality, social, eating disturbances, and respiratory scores); (2) G' versus peak flow, FVC, FEV_1 (before and after the Harvard step test), quality of life (vitality, social, eating disturbances, and respiratory scores); (3) G'' versus quality of life (vitality, social, eating disturbances, and respiratory scores); (4) pH versus VO_2, vitality score in quality of life (ESM 4). Small correlations could be evidence of alterations in rheology (beside being a minimum change) with inhaled use of $NaHCO_3$ that minimally alters the sputum pH for a brief period.

Spearman correlations between the values for dyspnea, peak flow, Harvard step test, spirometry, and quality of life and between the values for spirometry and quality of life of participants with CF during the protocol with inhaled bicarbonate can be seen, respectively, in ESM 5 and ESM 6. All positive correlations were small, and more studies

Table 2 Values for expectorated sputum pH, peak flow, and Harvard step test of participants with cystic fibrosis before (screening) and after inhaled bicarbonate protocols

pH of sputum Time collected	Screening[a]	Visit 1 (4.2% once a day)[a]	Visit 2 (4.2% twice daily)[a]	Visit 3 (8.4% once a day)[a]	Visit 4 (8.4% twice daily)[a]	Visit 5 (wash-out)[a]	*p* value
Baseline	7.333 ± 0.361	7.560 ± 0.492	7.888 ± 0.347	7.851 ± 0.341	7.999 ± 0.293	7.219 ± 0.382	**0.039*[c]**
30 min		7.417 ± 0.313	7.559 ± 0.289	7.469 ± 0.417	7.557 ± 0.284		0.375*
60 min		7.438 ± 0.270	7.426 ± 0.386	7.328 ± 0.241	7.317 ± 0.409		0.646*
90 min		7.338 ± 0.332	7.413 ± 0.399	7.370 ± 0.306	7.213 ± 0.351		0.878*
120 min		7.503 ± 0.390	7.248 ± 0.431	7.425 ± 0.375	7.235 ± 0.388		0.125*
150 min		7.341 ± 0.383	7.229 ± 0.499	7.401 ± 0.325	7.245 ± 0,0.395		0.466*
180 min		7.333 ± 0.257	7.257 ± 0.368	7.338 ± 0.308	7.257 ± 0.400		0.953*
p value		0.225*	0.090*	**0.004*[,d]**	**0.011*[,e]**		
Peak flow	398.75 ± 124.06	413.00 ± 114.97	427.08 ± 149.40	436.25 ± 136.70	405.42 ± 134.90	423.75 ± 147.14	**0.037*[f]**
Harvard step test							
Fitness	47.78 ± 6.24	48.68 ± 7.35	49.92 ± 8.38	45.22 ± 12.06	49.04 ± 7.01	49.35 ± 5.40	0.504*
VO_2 (oxygen consumption)[b]	121.24 ± 22.25	122.18 ± 22.94	122.18 ± 33.04	121.64 ± 41.94	127.38 ± 25.90	131.21 ± 27.32	0.112*
Work (energy expenditure)[b]	31.62 ± 8.73	32.30 ± 8.98	32.24 ± 10.69	32.46 ± 13.43	33.64 ± 9.77	34.78 ± 10.60	0.052*
Fitness	47.78 ± 6.24	48.22 ± 7.82				49.35 ± 5.40	0.417*
VO_2 (oxygen consumption)[b]	121.24 ± 22.25	123.35 ± 27.93				131.21 ± 27.32	**0.025*[g]**
Work (energy expenditure)[b]	31.62 ± 8.73	32.66 ± 10.20				34.78 ± 10.60	**0.024*[h]**

Visits: All visits occurred immediately after completing the indicated inhalation regimen. Each regime was conducted for a period of 2 weeks. Visit 1: aerosolized 4.2% $NaHCO_3$ inhaled once a day. Visit 2: 4.2% twice daily. Visit 3: 8.4% $NaHCO_3$, inhaled once a day. Visit 4: 8.4% $NaHCO_3$, inhaled twice daily, Visit 5: after 15 days of washout with no inhaled HCO_3^-

*Statistical analysis was performed by ANOVA with repeated measures by multivariate tests (Pillai's trace, Wilks' lambda, Hotelling's trace, and Roy's largest root), with post hoc pairwise analysis by the Bonferroni test. The covariance matrix of the transformed and orthonormalized dependent variable error was evaluated by Mauchly's sphericity test

**Statistical analysis performed by Friedman's two-way analysis of variance by paired sample stations. All participants were included in the evaluation. Significant *p* value is in bold. Alpha = 0.05

[a]Data given as mean ± SD

[b]Combined analysis—first line shows the arithmetic mean for the data collected during visits at 30-min intervals for 180 min. Second line shows mean for visits 1–4

[c]In the post-inhalation period, sputum pH collected during screening was significantly lower compared to visit 3 ($p = 0.026$) and visit 4 ($p = 0.02$). At visit 5, the pH value was significantly lower than the value observed at visits 2, 3, and 4 ($p = 0.011$, 0.050, 0.015, respectively)

[d]At visit 3, the first collection had a higher pH value compared to all others—30 min ($p = 0.001$), 60 min ($p = 0.002$), 90 min ($p = 0.008$), 120 min ($p = 0.034$), 150 min ($p = 0.015$), 180 min ($p = 0.021$)

[e]At visit 4, the first collection had a higher pH value when compared to later collections—30 min ($p = 0.003$), 60 min ($p = 0.004$), 90 min ($p = 0.001$), 120 min ($p = 0.004$), 150 min ($p = 0.001$), 180 min ($p = 0.003$)

[f]No difference was observed between the all-pairwise test groups by the Bonferroni method

[g]VO_2 was lower in the intervention period when compared to the post-intervention period ($p = 0.030$)

[h]Work was higher after the intervention period when compared to the period during the intervention ($p = 0.030$)

need to be carried out to confirm these exploratory findings (Tables 4, 5).

No changes were observed in blood count, venous blood gas, respiratory rate, heart rate, SpO_2, body temperature, or incidence of dyspnea.

Importantly, no adverse events associated with the study were observed.

4 Discussion

This in vivo clinical study assesses the safety, tolerability, and effects of $NaHCO_3$ inhalation in subjects with CF. The pH and rheological properties of sputum as well as clinical/laboratory data were evaluated before, during, and

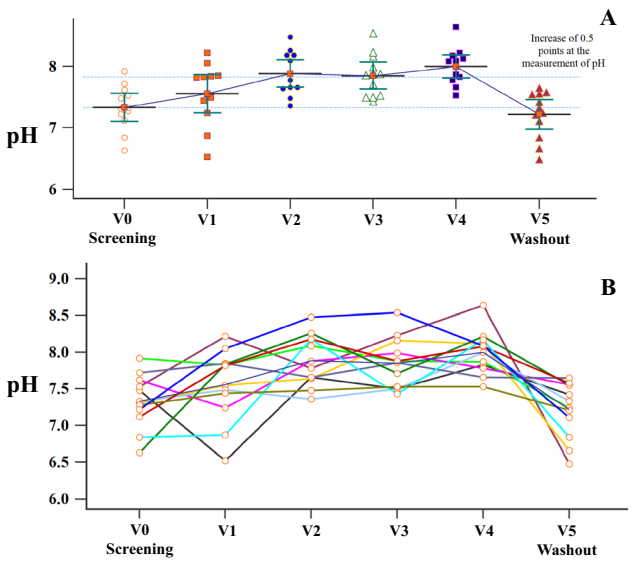

Fig. 1 **a** pH of induced sputum of participants with cystic fibrosis during the period of intervention with inhaled bicarbonate aerosol. The data show as a marker in the figure the increase of 0.5 pH units during the intervention with 8.4% $NaHCO_3$. The complete data are shown in Table 2. **b** Change in each individual's sputum pH at baseline measurement with trend lines. Screening visit; (V1) 4.2% (once a day) over 2 weeks; (V2) 4.2% (twice daily) over 2 weeks; (V3) 8.4% (twice daily) over 2 weeks; (V4) 8.4% (twice daily) over 2 weeks; (V5) no drug—wash-out period. In the post-inhalation period, sputum pH collected during screening was significantly lower compared to visit 3 ($p=0.026$) and visit 4 ($p=0.02$). At visit 5 (washout), the pH value was significantly lower than the values observed at visits 2, 3, and 4 ($p=0.011$, 0.050, 0.015, respectively). Statistical analysis was performed by ANOVA with repeated measures by multivariate tests (Pillai's trace, Wilks' lambda, Hotelling's trace, and Roy's largest root), with post hoc pairwise analysis by the Bonferroni test. The covariance matrix of the transformed and orthonormalized dependent variable error was evaluated by Mauchly's sphericity test. All participants were included in the evaluation. Alpha = 0.05

after interventions with inhaled aerosols of two concentrations (4.2% and 8.4%) of solutions of $NaHCO_3$ with a dose escalation every 2 weeks for eight consecutive weeks.

The data show that inhaled $NaHCO_3$ acutely raised the pH of sputum, which correlated well with all rheological moduli (G', G'', and G^*). In addition, other markers, including quality of life and the Harvard step test, improved significantly during the study intervention period.

$NaHCO_3$ is essential for proper expansion and maturation of mucin granules [8, 11, 16]. In addition, mucin granules are influenced by many factors, such as hydration, pH, solution ionic strength, composition, concentration, electrostatic interactions between mucins, size of polymers, degree of cross-linking, debris, etc. [25].

In a study on the effect of mixing $NaHCO_3$ solution directly with the sputum of CF patients in vitro, Stigliani et al. [15] reported reductions in rheological moduli G', G'', and G^*. However, the present study evaluated native expectorated sputum. In the samples from the same participant and among the participants, quantitative/qualitative macroscopic variability was observed, particularly in the volume of expectorations. The large variability in properties between individual sputum specimens will likely require a much larger population to firmly establish statistically significant changes resulting from inhaled $NaHCO_3$ aerosols. Nonetheless, in the present small population, the results between screening V0 and after washout V5 often indicated a statistically significant change in sputum properties such as pH at visits V2–V4 that may be clinically important for future studies.

The variability of sputum rheology is multifactorial, including physicochemical aspects, and may be affected by: (1) repetitive voluntary cough, which may change the solid content and alter mucus hydration in the airways; (2) surface tension; (3) surface contact time with the respiratory airways; (4) adherence to bronchial wall [26]. The most direct method to obtain sputum may be bronchoscopy with bronchoalveolar lavage; however, due to ethical considerations and the probable change in properties of the mucus as a consequence of the saline wash to remove the native sputum, this method was not used [19]. The bicarbonate solution is hyperosmotic and may draw fluid into the airway surface liquids (ASL) as is thought to occur with hypertonic saline, thereby "thinning" the ASL and its contents. Again, like hypertonic saline, it may act as a minor irritant and stimulate airways secretions too.

Although native expectorated sputum was evaluated, the gel component of sputum in rheological analysis is critically important. Recent studies [27, 28] describe a positive correlation of the gel component with the G', G'', and G^* moduli. Here, liquids and saliva largely separated spontaneously from the gel component of the sputum when compressed between the rheometer plates supporting the presumption that measurements principally reflect gel (mucus) properties. Rheological differences in sputum from patients with CF, chronic obstructive pulmonary disease, and acute pulmonary infections have been reported [19]. During pulmonary exacerbations, elasticity and viscosity are increased, and return to basal levels only after antibiotic therapy along with normalization of pulmonary function [29].

An important finding herein is that an increase in sputum pH immediately accompanies $NaHCO_3$ inhalation. Lower pH of ASL favors bacterial growth. A recent study in a porcine CF model found the growth of micro-organisms in CF was enhanced significantly in reduced extracellular fluid pH resulting from decreased secretion of HCO_3^- [13]. Presently, we are not able to establish that higher pH associated with the interventions here inhibited bacterial growth; further studies are needed for confirmation.

Table 3 Quality-of-life scores from subjects with cystic fibrosis during the protocol with inhaled bicarbonate

Marker[a]	Screening	Visit 1 (4.2% once a day)[b]	Visit 2 (4.2% twice daily)[b]	Visit 3 (8.4% once a day)[b]	Visit 4 (8.4% twice daily)[b]	Visit 5 (wash-out)[b]	*p* value*
Physical functioning	52.05 (38.53–84.30)	58.30 (40.55–82.28)	58.15 (50.00–81.25)	60.25 (42.65–70.80)	60.40 (46.85 to 89.55)	56.20 (43.70 to 85.35)	0.499
Physical role	83.30 (75.00–97.90)	91.60 (70.78–97.80)	91.60 (91.60–100)	83.30 (66.60–100)	91.60 (77.08 to 100)	84.45 (83.30 to 97.90)	0.146
Vitality	58.30 (50.00–72.90)	66.60 (51.38–75.00)	66.60 (52.08–75.00)	66.60 (52.08–75.00)	75.00 (50.00 to 83.30)	70.80 (52.80 to 83.30)	0.452
Emotional functioning	73.30 (60.00–85.78)	80.00 (60.00–84.95)	76.65 (61.65–82.48)	80.00 (61.65–86.60)	76.65 (61.65 to 91.63)	73.30 (60.00 to 85.78)	0.630
Social	58.30 (50.00–77.70)	66.60 (50.00–81.90)	69.40 (52.88–87.43)	74.95 (51.38–88.80)	66.60 (51.38 to 86.03)	69.40 (45.80 to 81.90)	0.461
Body image	61.05 (33.30–97.20)	66.60 (44.40–100)	66.60 (47.18–100)	66.60 (27.75–100)	72.15 (36.08 to 100)	72.15 (30.53 to 100)	0.775
Eating disturbances	100 (77.70–100)	94.40 (77.70–100)	100 (83.28–100)	100 (100–100)	100 (100 to 100)	100 (77.70 to 100)	0.312
Treatment burden	44.40 (36.08–66.60)	55.50 (36.08–77.70)	55.50 (36.08–77.03)	55.50 (36.08–66.60)	55.50 (33.30 to 66.60)	55.50 (55.50 to 66.60)	0.131
Health perceptions	44.40 (33.30–55.50)	44.40 (33.30–66.60)	61.05 (36.08–74.93)	61.05 (36.08–82.95)	61.05 (27.75 to 66.60)	49.95 (36.08 to 82.03)	**0.011**
Weight	16.65 (0–100)	66.60 (8.33–100)	66.60 (33.30–100)	83.30 (8.33–100)	49.95 (0 to 100)	66.60 (8.33 to 100)	0.246
Respiratory symptoms	66.60 (56.90–66.60)	63.85 (55.53–72.20)	66.60 (61.10–74.30)	72.20 (58.28–72.20)	63.85 (55.50 to 72.20)	69.40 (61.10 to 77.70)	0.829
Digestive symptoms	88.80 (77.70–100)	100 (80.48–100)	100 (77.70–100)	100 (72.15–100)	100 (80.48 to 100)	77.70 (66.60 to 100)	0.203
Total	64.86 (56.04–73.76)	67.78 (63.90–78.20)	73.14 (67.66–73.14)	70.77 (62.35–82.30)	72.20 (65.14 to 79.12)	72.20 (58.04 to 82.12)	**0.027**

Marker[a]	Screening	Visits 1–4	Visit 5	*p* value*
Physical functioning	52.05 (38.53–84.30)	57.19 (46.86–79.92)	56.20 (43.70–85.35)	0.266
Physical role	83.30 (75.00–97.90)	89.55 (80.17–96.85)	84.45 (83.30–97.90)	0.284
Vitality	58.30 (50.00–72.90)	67.66 (52.94–76.53)	70.80 (52.80–83.30)	0.266
Emotional functioning	73.30 (60.00–85.78)	78.31 (60.40–83.91)	73.30 (60.00–85.78)	0.931
Social	58.30 (50.00–77.70)	67.28 (53.18–86.38)	69.40 (45.80–81.90)	0.377
Body image	61.05 (33.30–97.20)	70.76 (38.16–99.30)	72.15 (30.53–100)	0.670
Eating disturbances	100 (77.70–100)	98.60 (92.33–100)	100 (77.70–100)	0.834
Treatment burden	44.40 (36.08–66.60)	56.89 (42.32–69.21)	55.50 (55.50–66.60)	**0.010**
Health perceptions	44.40 (33.30–55.50)	56.89 (33.30–69.38)	49.95 (36.08–82.03)	**0.030**
Weight	16.65 (0–100)	54.13 (20.81–100)	66.60 (8.33–100)	0.228
Respiratory symptoms	66.60 (56.90–66.60)	65.24 (60.57–71.50)	69.40 (61.10–77.70)	0.502
Digestive symptoms	88.80 (77.70–100)	94.43 (83.28–100)	77.70 (66.60–100)	**0.015**
Total	64.86 (56.04–73.76)	71.97 (66.60–78.39)	72.20 (58.04–82.12)	**0.009**

*Statistical analysis performed by Friedman's two-way analysis of variance by paired sample stations. In the comparison with the pairwise method between groups with significant *p* values (*p* values adjusted for multiple comparisons): *treatment*—the score was lower before intervention when compared to the post-intervention period ($p=0.018$); *health*—in the simultaneous analysis for all groups, no significant association was observed; however, in grouped data, a lower score was obtained before the intervention compared to the post-intervention period ($p=0.043$); *digestive*—no significant association in the analysis; *Overall total* in the simultaneous analysis for all groups the score was significantly lower before the intervention compared to the post-intervention period ($p=0.034$). The analysis for the grouped data similarly was significantly different ($p=0.007$). Significant *p* values are in bold. Alpha = 0.05

[a]*Combined analysis* gives the arithmetic mean for the data collected from all subjects during each separate visit, then the arithmetic mean of all visits after regimens one to four were calculated together

[b]*Visits*: Each visit occurred after 2 weeks on each regime indicated. Visit 1: 4.2% $NaHCO_3$ aerosol was inhaled once a day. Visit 2: 4.2% $NaHCO_3$, aerosol was inhaled twice daily; Visit 3: 8.4% $NaHCO_3$, aerosol was inhaled once a day; Visit 4: 8.4% $NaHCO_3$ aerosol was inhaled twice daily. After last day of 8.4% inhalations, no $NaHCO_3$ was inhaled during the 15 days before the evaluation at Visit 5. Data presented as medians (25th percentile–75th percentile)

Table 4 Microbiological characterization of sputum from participants with cystic fibrosis before and after the intervention period with inhaled sodium bicarbonate

Patient	Non-mucoid *Pseudomonas aeruginosa*		Mucoid *P. aeruginosa*		*Staphylococcus aureus*	
	Pre	Post	Pre	Post	Pre	Post
P2	++	+	++	++		
P3					++	++
P5	++	+	++	+	+++	++
P6			+++	++		
P8	++	+++	++	+++		
P10	+	++	+	++	++	++
P12		+++	+++	+++		
P13					+++	++
P15		+	++	+	+	
P17		+	+	+	+++	+
P18			++	+	+++	+
P19					+++	++

A semi-quantitative analysis of bacterial content in the sputum specimens was performed at the initial screening visit (pre) and at the final visit 5 (post). The bacterial load was classified as: + light; ++ moderate; +++ heavy. The *S. aureus* load was reduced in five participants; mucoid *P. aeruginosa* was reduced in four participants, and non-mucoid *P. aeruginosa* was reduced in two participants. However, five participants and two participants showed an increased bacterial load with non-mucoid *P. aeruginosa* and mucoid *P. aeruginosa,* respectively. Overall, bacteria decreased in 11 assays, increased in seven and remained unchanged in 17 assays

Table 5 Spearman correlation between pH and rheology values for all sputum specimens analyzed ($n = 360$) between and during all visits of patients with cystic fibrosis during the inhalation regimens with inhaled bicarbonate

		G^*	G'	G''	G'- G''
pH	Correlation coefficient	− 0.241	− 0.287	− 0.275	− 0.214
	p value	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**

Significant *p* value in bold. Alpha = 0.05

In healthy subjects, the pH of ASL was reported to range from 6.85 to 7.65 [30, 31], but in CF, the range was reported to be 4.5–8.5 [31]. In the present study, $NaHCO_3$ inhalation raised the sputum pH significantly in all visits and to ~ 7.9 in the last three visits, which is higher than the maximum value reported for healthy subjects (7.65). The duration of the raised pH effect of the inhalation (which required about 15–20 min to inhale nebulized the dose) was approximately 150 min. Therefore, increasing the frequency of $NaHCO_3$ inhalations should be tested (every 4–6 h dosing); considering that most patients are prescribed airway clearance therapy two to three times per day, more frequent bicarbonate dosing may be feasible, but a search for alternative formulations that might allow a longer duration of action seems justifiable. A corroborating increase in pH due to $NaHCO_3$ in vitro has been described [15].

The most favorable pH for growth and maintenance of bacterial colonization/infection in CF lungs is uncertain. However, it is commonly accepted that in CF a low volume of ASL and/or its acidity due to lack of HCO_3^- changes mucociliary clearance and favors pulmonary infection [1, 32–34]. Recent findings of acidity on the airway luminal epithelium in CF affirms the development of poor mucociliary clearance and infection [8, 13, 35, 36]. In contrast, others have reported that airway pH in CF is not different from that of healthy subjects [37, 38]. In short, bacteria decreased in 11 assays, increased in seven assays, and remained unchanged in 17 assays.

Using FEV_1 as a principal clinical marker, higher viscoelasticity of mucus appears to contribute to pulmonary obstruction and reduced lung function [39]. This marker was followed in multicenter studies of inhaled hypertonic saline and dornase alpha to demonstrate FEV_1 increased with these drugs [40]. Although the present study did not reach statistical significance, two participants had improved FEV_1 after inhaling $NaHCO_3$ (data not shown); thus, for a more compelling analysis of FEV_1 responses, a larger sample size and follow-up study, possibly for a longer duration of treatment, are required.

Quality of life is a marker widely used in intervention studies for new drugs as it provides personalized patient input [41, 42]. As a consequence of the results considered above, a cascade of events may occur that affect the quality of life. Lower pH changes the immune response in airways, and increases bacterial colonization favoring biofilm formation with a consequent increase in inflammation/infection, airway obstruction, and worsening of quality of life

[43–45]. Of note, the highest rheology values (increased viscoelasticity) were associated with worsening dyspnea, but correlations were weak.

Low tolerability to physical exercise is a common effect of CF, which increases with disease severity and is related to a higher mortality rate [46]. The Harvard step test is an accepted tool to evaluate tolerability to physical exercise [47]. Higher values of this measure related to a lower incidence of cardiopulmonary disease and better predisposition or physical activity [48, 49]. Here, the Harvard step test showed an improvement in oxygen consumption and energy expenditure after the intervention with inhaled $NaHCO_3$ (Table 2). In spite of being an exploratory analysis, our data indicated an improvement in both oxygen consumption and energy expenditure that appeared to be related to the intervention and could be associated with a better capacity to perform daily life activities with inhaled $NaHCO_3$.

In brief, as reported in Sect. 2, an exacerbation was observed during the first period of inhalation in two patients and was noted as a clinical finding during the first visit, V1. An incidence of exacerbations in patients with CF might be expected to be greater than two of 19 potential subjects in this timeframe. Thus, it seems reasonable that these events preceded the intervention and were not caused by it. Consequently, both patients were excluded from the analysis and no data were collected from these patients. It is important to note that no further incidence of exacerbations occurred at any time in any of the remaining participants during the 10 weeks of the study.

4.1 Study Limitations

The apparent limitations of this study include: (1) inadequate sample size to confirm or reject correlations and establish intervention effects. However, a recent study of 12 patients with CF showed that acute and longitudinal changes in lung function correlated with mucus rheology in spite of very heterogenous parameters among the patients [29]. (2) Suboptimal sputum separation (saliva, mucus gel, air) to perform precision rheological assays. (3) Lack of a placebo and/or active control group (e.g., hypertonic saline). (4) Short-term follow-up for a more robust identification of clinical markers such as lung function. (5) Older patients with more severe lung disease that may obscure lung function improvements, in view of likely irreversible damage to airways and lung parenchyma. (6) Alkalization of ASL may impede bacteria growth; therefore, we collected specimens for culture only after V5, the fifth and final visit (wash-out). It seems possible that sputum cultures at the end of V4 would have been more appropriate for evaluating acute versus longer term microbiological effects. (7) During the trial interventions we did not collect blood for complete blood counts or venous blood gas.

The study limitations might be reduced by: (1) increasing the sample size and follow-up time with the inclusion of more than one center. (2) Selecting participants without extremely severe disease; including younger participants with less severe airway pathology. (3) Further exploration for optimal intervention duration, dose concentration, and inhalation frequency for $NaHCO_3$ (dose-escalation study). (4) Evaluating mucins and inflammatory markers in fresh, native sputum at the time of collection.

5 Conclusion

This study documents that inhalation of aerosolized $NaHCO_3$ is safe and very well tolerated by CF participants. There were no significant adverse events associated with the interventions, even at the highest dosage of 5 mL saturated solution of $NaHCO_3$ (8.4%) inhaled twice daily. However, due to the small number of participants and inherently wide range in values of measured parameters, for example, sputum specimen (rheology/pH), spirometry, fitness, microbiology, and other variables in the population, the effects of the intervention merit further studies in a larger number of subjects to reveal and establish the potential therapeutic benefits that are suggested here. The apparent benefits of inhaled $NaHCO_3$ are due most likely to its effects on raising the pH of the ASL and on improving sputum rheology as a function of more fully expanded mucin and DNA macromolecules in the presence of higher concentrations of HCO_3^- in the ASL. These changes would enhance the respiratory immune defense and favor improved mucociliary clearance of the airways in maintaining improved airway hygiene.

In conclusion, even though most measured parameters did not achieve statistical significance, the means of the measurements of effects almost always remained constant or changed in the direction of presumed improvement, giving confidence that the treatment did not depress or impede normal function. The favorable changes observed in several parameters in this study may support inhalation of nebulized $NaHCO_3$ as a safe and well-tolerated therapeutic agent in the management of CF and possibly other obstructive lung diseases.

Author Contributions All authors approved the manuscript and agreed with its submission. CCSG formalized the protocol, collected patient data, wrote the manuscript, and responded to critical reviews of the study; FALM performed the statistical analysis of the data, wrote the manuscript, critically reviewed the study, and responded to critical reviews of the study; PFLP, KJC, RMM, FBTP, and MAGOR collected data; DC and PMQ provided the project concept and edited the manuscript; AOP, CEL, and AFR provided supervision and contributed to the safety analysis; JDR worked on supervision and the project concept, clinically evaluated the patients included in the study, and validated phenotypic findings according to repeatability criteria. All authors contributed to writing the manuscript.

Compliance with Ethical Standards

Funding FALM and JDR Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for #2011/12939-4, #2011/18845-1, #2015/12183-8 and #2015/12858-5.

Conflicts of interest The authors declare they have no conflicts of interest.

Ethical approval All procedures in this study were in accordance with the 1964 Helsinki Declaration and its amendments. The project (#12398956) was approved by the research ethics committee of the Faculdade de Ciências Médicas, Universidade Estadual de Campinas.

Informed consent Informed consent was obtained from each participant.

References

1. Quinton PM. Both ways at once: keeping small airways clean. Physiology (Bethesda). 2017;32(5):380–90.
2. Benedetto R, Ousingsawat J, Wanitchakool P, Zhang Y, Holtzman MJ, Amaral M, Rock JR, Schreiber R, Kunzelmann K. Epithelial chloride transport by CFTR requires TMEM16A. Sci Rep. 2017;7(1):12397.
3. Henderson AG, Ehre C, Button B, Abdullah LH, Cai LH, Leigh MW, DeMaria GC, Matsui H, Donaldson SH, Davis CW, Sheehan JK, Boucher RC, Kesimer M. Cystic fibrosis airway secretions exhibit mucin hyperconcentration and increased osmotic pressure. J Clin Invest. 2014;124(7):3047–60.
4. Kunzelmann K, Schreiber R, Hadorn HB. Bicarbonate in cystic fibrosis. J Cyst Fibros. 2017;16(6):653–62.
5. Muchekehu RW, Quinton PM. A new role for bicarbonate secretion in cervico-uterine mucus release. J Physiol. 2010;588(Pt 13):2329–42.
6. Lee MG, Ohana E, Park HW, Yang D, Muallem S. Molecular mechanism of pancreatic and salivar gland fluid and HCO_3 secretion. Physiol Rev. 2012;92(1):39–74.
7. Wine JJ, Hansson GC, König P, Joo NS, Ermund A, Pieper M. Progress in understanding mucus abnormalities in cystic fibrosis airways. J Cyst Fibros. 2018;17(2S):S35–9.
8. Quinton PM. Cystic fibrosis: impaired bicarbonate secretion and mucoviscidosis. Lancet. 2008;372(9636):415–7.
9. Garcia MA, Yang N, Quinton PM. Normal mouse intestinal mucus release requires cystic fibrosis transmembrane regulator-dependent bicarbonate secretion. J Clin Invest. 2009;119(9):2613–22.
10. Yang N, Garcia MA, Quinton PM. Normal mucus formation requires cAMP-dependent HCO_3^- secretion and Ca^{2+}-mediated mucin exocytosis. J Physiol. 2013;591(18):4581–93.
11. Gustafsson JK, Ermund A, Ambort D, Johansson ME, Nilsson HE, Thorell K, Hebert H, Sjövall H, Hansson GC. Bicarbonate and functional CFTR channel are required for proper mucin secretion and link cystic fibrosis with its mucus phenotype. J Exp Med. 2012;209(7):1263–72.
12. Ermund A, Meiss LN, Rodriguez-Pineiro AM, Bahr A, Nilsson HE, Trillo-Muyo S, Ridley C, Thornton DJ, Wine JJ, Hebert H, Klymiuk N, Hansson GC. The normal trachea is cleaned by MUC5B mucin bundles from the submucosal glands coated with the MUC5AC mucin. Biochem Biophys Res Commun. 2017;492(3):331–7.
13. Pezzulo AA, Tang XX, Hoegger MJ, Abou Alaiwa MH, Ramachandran S, Moninger TO, Karp PH, Wohlford-Lenane CL, Haagsman HP, van Eijk M, Bánfi B, Horswill AR, Stoltz DA, McCray PB Jr, Welsh MJ, Zabner J. Reduced airway surface pH impairs bacterial killing in the porcine cystic fibrosis lung. Nature. 2012;487(7405):109–13.
14. Tiddens HA, Donaldson SH, Rosenfeld M, Pare PD. Cystic fibrosis lung disease starts in the small airways: can we treat it more effectively? Pediatr Pulmonol. 2010;45(2):107–17.
15. Stigliani M, Manniello MD, Zegarra-Moran O, Galietta L, Minicucci L, Casciaro R, Garofalo E, Incarnato L, Aquino RP, Del Gaudio P, Russo P. Rheological properties of cystic fibrosis bronchial secretion and in vitro drug permeation study: the effect of sodium bicarbonate. J Aerosol Med Pulm Drug Deliv. 2016;29(4):337–45.
16. Chen EY, Yang N, Quinton PM, Chin WC. A new role for bicarbonate in mucus formation. Am J Physiol Lung Cell Mol Physiol. 2010;299(4):542–9.
17. Tarran R, Donaldson S, Boucher RC. Rationale for hypertonic saline therapy for cystic fibrosis lung disease. Semin Respir Crit Care Med. 2007;28(3):295–302.
18. Rasgado-Flores H, Krishna Mandava V, Siman H, Van Driessche W, Pilewski JM, Randell SH, Bridges RJ. Effect of apical hyperosmotic sodium challenge and amiloride on sodium transport in human bronchial epithelial cells from cystic fibrosis donors. Am J Physiol Cell Physiol. 2013;305(11):C1114–22.
19. Serisier DJ, Carroll MP, Shute JK, Young SA. Macrorheology of cystic fibrosis, chronic obstructive pulmonary disease and normal sputum. Respir Res. 2009;10:63.
20. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J, ATS/ERS Task Force. Standardisation of spirometry. Eur Respir J. 2005;26(2):319–38.
21. Measuring your peak flow rate. American Lung Association. https://www.lung.org/lung-health-and-diseases/lung-disease-lookup/asthma/living-with-asthma/managing-asthma/measuring-your-peak-flow-rate.html.
22. Quittner AL, Buu A, Messer MA, Modi AC, Watrous M. Development and validation of The Cystic Fibrosis Questionnaire in the United States: a health-related quality-of-life measure for cystic fibrosis. Chest. 2005;128(4):2347–54.
23. Eakin EG, Resnikoff PM, Prewitt LM, Ries AL, Kaplan RM. Validation of a new dyspnea measure: the UCSD shortness of breath questionnaire. University of California, San Diego. Chest. 1998;113(3):619–24.
24. Marson FAL, Bertuzzo CS, de Araujo TK, Hortencio TDR, Ribeiro AF, Ribeiro JD. Pancreatic insufficiency in cystic fibrosis: influence of inflammatory response genes. Pancreas. 2018;47(1):99–109.
25. Lai SK, Wang YY, Wirtz D, Hanes J. Micro- and macrorheology of mucus. Adv Drug Deliv Rev. 2009;61(2):86–100.
26. Cone RA. Barrier properties of mucus. Adv Drug Deliv Rev. 2009;61(2):75–85.
27. Hill DB, Vasquez PA, Mellnik J, McKinley SA, Vose A, Mu F, Henderson AG, Donaldson SH, Alexis NE, Boucher RC, Forest MG. A biophysical basis for mucus solids concentration as a candidate biomarker for airways disease. PLoS One. 2014;9(2):e87681.
28. Duncan GA, Jung J, Joseph A, Thaxton AL, West NE, Boyle MP, Hanes J, Suk JS. Microstructural alterations of sputum in cystic fibrosis lung disease. JCI Insight. 2016;1(18):e88198.
29. Ma JT, Tang C, Kang L, Voynow JA, Rubin BK. Cystic fibrosis sputum rheology correlates with both acute and longitudinal changes in lung function. Chest. 2018;154(2):370–7.
30. Hunt JF, Fang K, Malik R, Snyder A, Malhotra N, Platts-Mills TA, Gaston B. Endogenous airway acidification. Implications for

asthma pathophysiology. Am J Respir Crit Care Med. 2000;161(3 Pt 1):694–9.
31. Fisher H. Function of proton channels in lung epithelia. Wiley Interdiscip Rev Membr Transp Signal. 2012;1(3):247–58.
32. Quinton PM. Role of epithelial HCO_3-transport in mucin secretion lessons from cystic fibrosis. Am J Physiol Cell Physiol. 2010;299(6):C1222–33.
33. Button B, Cai LH, Ehre C, Kesimer M, Hill DB, Sheehan JK, Boucher RC, Rubinstein M. A periciliary brush promotes the lung health by separating the mucus layer from airway epithelia. Science. 2012;337(6097):937–41.
34. Luan X, Belev G, Tam JS, Jagadeeshan S, Hassan N, Gioino P, Grishchenko N, Huang Y, Carmalt JL, Duke T, Jones T, Monson B, Burmester M, Simovich T, Yilmaz O, Campanucci VA, Machen TE, Chapman LD, Ianowski JP. Cystic fibrosis swine fail to secrete airway surface liquid in response to inhalation of pathogens. Nat Commun. 2017;8(1):786.
35. Coakley RD, Grubb BR, Paradiso AM, Gatzy JT, Johnson LG, Kreda SM, O'Neal WK, Boucher RC. Abnormal surface liquid PH regulation by cultured cystic fibrosis bronchial epithelium. Proc Natl Acad Sci USA. 2003;100(26):16083–8.
36. Hisert KB, Heltshe SL, Pope C, Jorth P, Wu X, Edwards RM, Radey M, Accurso FJ, Wolter DJ, Cooke G, Adam RJ, Carter S, Grogan B, Launspach JL, Donnelly SC, Gallagher CG, Bruce JE, Stoltz DA, Welsh MJ, Hoffman LR, McKone EF, Singh PK. Restoring cystic fibrosis transmembrane conductance regulator function reduces airway bacteria and inflammation in people with cystic fibrosis and chronic lung infections. Am J Respir Crit Care Med. 2017;195(12):1617–28.
37. McShane D, Davies JC, Davies MG, Bush A, Geddes DM, Alton EW. Airway surface pH in subjects with cystic fibrosis. Eur Respir J. 2003;21(1):37–42.
38. Schultz A, Puvvadi R, Borisov SM, Shaw NC, Klimant I, Berry LJ, Montgomery ST, Nguyen T, Kreda SM, Kicic A, Noble PB, Button B, Stick SM. Airway surface liquid pH is not acidic in children with cystic fibrosis. Nat Commun. 2017;8(1):1409.
39. Tomaiuolo G, Rusciano G, Caserta S, Carciati A, Carnovale V, Abete P, Sasso A, Guido S. A new method to improve the clinical evaluation of cystic fibrosis patients by mucus viscoelastic properties. PLoS ONE. 2014;9(1):e82297.
40. Suri R, Wallis C, Bush A, Thompson S, Normand C, Flather M, Grieve R, Metcalfe C, Lees B. A comparative study of hypertonic saline, daily and alternate-day rhDNase in children with cystic fibrosis. Health Technol Assess. 2002;6(34):1–60.
41. Quittner AL, Abbott J, Georgiopoulos AM, Goldbeck L, Smith B, Hempstead SE, Marshall B, Sabadosa KA, Elborn S, International Committee on Mental Health, EPOS Trial Study Group. International Committee on mental health in cystic fibrosis: cystic fibrosis foundation and European cystic Fibrosis Society consensus statements for screening and treating depression and anxiety. Thorax. 2016;71(1):26–34.
42. Hammond JA, Connett GJ. The use of lumacaftor/ivacaftor to treat acute deterioration in paediatric cystic fibrosis. Paediatr Respir Rev. 2018;27:16–7.
43. Starner TD, Zhang N, Kim G, Apicella MA, McCray PB Jr. Haemophilus influenzae forms biofilms on airway epithelia: implications in cystic fibrosis. Am J Respir Crit Care Med. 2006;174(2):213–20.
44. Bjarnsholt T, Jensen PØ, Fiandaca MJ, Pedersen J, Hansen CR, Andersen CB, Pressler T, Givskov M, Høiby N. *Pseudomonas aeruginosa* biofilms in the respiratory tract of cystic fibrosis patients. Pediatr Pulmonol. 2009;44(6):547–58.
45. Høiby N, Ciofu O, Bjarnsholt T. *Pseudomonas aeruginosa* biofilms in cystic fibrosis. Future Microbiol. 2010;5(11):1663–74.
46. Pianosi P, LeBlanc J, Almudevar A. Relationship between FEV_1 and peak oxygen uptake in children with cystic fibrosis. Pediatr Pulmonol. 2005;40(4):324–9.
47. Ferrazza AM, Martolini D, Valli G, Palange P. Cardiopulmonary exercise testing in the functional and prognostic evaluation of patients with pulmonary diseases. Respiration. 2009;77(1):3–17.
48. Arena R, Myers J, Abella J, Peberdy MA, Pinkstaff S, Bensimhon D, Chase P, Guazzi M. Prognostic value of timing and duration characteristics of exercise oscillatory ventilation in patients with heart failure. J Heart Lung Transplant. 2008;27(3):341–7.
49. Cornelis J, Taeymans J, Hens W, Beckers P, Vrints C, Vissers D. Prognostic respiratory parameters in heart failure patients with and without exercise oscillatory ventilation a systematic review and descriptive meta-analysis. Int J Cardiol. 2015;182:476–86.

Affiliations

Carla Cristina Souza Gomez[1] · Paloma Lopes Francisco Parazzi[1] · Karl Jan Clinckspoor[2] · Renan Marrichi Mauch[1] · Francisco Benedito Teixeira Pessine[2] · Carlos Emilio Levy[3] · Andressa Oliveira Peixoto[1] · Maria Ângela Gonçalves Oliveira Ribeiro[1] · Antônio Fernando Ribeiro[1] · Douglas Conrad[4] · Paul Marquis Quinton[5] · Fernando Augusto Lima Marson[1,6,7] · José Dirceu Ribeiro[1]

Paloma Lopes Francisco Parazzi
palomaparazzi@yahoo.com.br

Karl Jan Clinckspoor
karl970@gmail.com

Renan Marrichi Mauch
renanmauch@gmail.com

Francisco Benedito Teixeira Pessine
fpessine@iqm.unicamp.br

Carlos Emilio Levy
levy.carlosemilio@gmail.com

Andressa Oliveira Peixoto
andressa_op@hotmail.com

Maria Ângela Gonçalves Oliveira Ribeiro
ribeiromago@gmail.com

Antônio Fernando Ribeiro
anferi@uol.com.br

Douglas Conrad
dconrad@ucsd.edu

Paul Marquis Quinton
pquinton@ucsd.edu

[1] Center for Investigation in Pediatrics, Department of Pediatrics, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, 126, Tessália Vieira de Camargo, Campinas, São Paulo 13083-887, Brazil

[2] Department of Physical Chemistry, Instituto de Química, Universidade Estadual de Campinas, 336, Josué de Castro, Campinas, São Paulo 13083-970, Brazil

[3] Department of Clinical Pathology, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, 126, Tessália Vieira de Camargo, Campinas, São Paulo 13083-887, Brazil

[4] Division of Pulmonary, Critical Care and Sleep Medicine, University of California, San Diego School of Medicine, 9500 Gilman Dr., La Jolla, CA 92093-0830, USA

[5] Department of Pediatrics, University of California, San Diego School of Medicine, 9500 Gilman Dr, La Jolla, CA 92093-0830, USA

[6] Department of Medical Genetics and Genomic Medicine, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, 126, Tessália Vieira de Camargo, Campinas, São Paulo 13083-887, Brazil

[7] Postgraduate Program in Health Science, Laboratory of Medical and Human Genetics, São Francisco University, Avenida São Francisco de Assis 218, Bragança Paulista, São Paulo 12916-900, Brazil