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Short Communication

Neonates with cystic fibrosis have a reduced nasal liquid pH; A small pilot study

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Abstract

Background: Disrupted HCO_3^- transport and reduced airway surface liquid (ASL) pH in cystic fibrosis (CF) may initiate airway disease. We hypothesized that ASL pH is reduced in neonates with CF.
Methods: In neonates with and without CF, we measured pH of nasal ASL. We also measured nasal pH in older children and adults.
Results: In neonates with CF, nasal ASL (pH 5.2 ± 0.3) was more acidic than in non-CF neonates (pH 6.4 ± 0.2). In contrast, nasal pH of CF children and adults was similar to values measured in people without CF.
Conclusions: At an age when infection, inflammation and airway wall remodeling are minimal, neonates with CF had an acidic nasal ASL compared to babies without CF. The CF:non-CF pH difference disappeared in older individuals, perhaps because secondary manifestations of disease increase ASL pH. These results aid understanding of CF pathogenesis and suggest opportunities for therapeutic intervention and monitoring of disease.

Keywords: Cystic fibrosis; Neonates; pH; Airway surface liquid (ASL); Neonatal screen

1. Introduction

Cystic fibrosis (CF) is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel [1,2]. Airway disease characterized by bacterial infection and inflammation remains the major cause of morbidity and mortality.

CFTR channels conduct both Cl^- and HCO_3^- [3]. Several observations suggest that reduced HCO_3^- transport may be a key factor in the pathogenesis of CF airway disease. First, loss of CFTR impairs HCO_3^- secretion across airway epithelia cultured from humans [4,5] and pigs with a disrupted *CFTR* gene [6]; CF pigs spontaneously develop lung disease that mimics human CF [7]. Second, loss of CFTR reduces the pH of airway surface liquid (ASL) in cultured human airway epithelia [5], of secretions from human submucosal glands studied *ex vivo* [8], and of ASL studied *in vivo*, *ex vivo*, and in epithelial cultures from CF pigs [9]. Third, a reduced pH decreases the activity of antimicrobials in ASL *in vivo* and *in vitro*, thereby

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impairing eradication of bacteria that land on the airway surface [9]. Fourth, a reduced pH may alter the properties of mucus secreted into the airways and thereby hinder mucociliary transport [10].

However, an earlier study reported that there was no CF: non-CF difference in ASL pH measured *in vivo* in humans [11]. That result contrasts with the observation that airway pH is reduced in the ASL of CF pigs studied within 24 h of birth [9]. That discrepancy might be due to differences in the state of the airways because the humans with CF were studied as adults or older children, a time when airway disease is present [11]. In contrast, newborn CF pigs lack airway infection, inflammation, goblet cell hyperplasia and submucosal gland hypertrophy [7].

Therefore, we hypothesized that like newborn CF pigs, ASL in human neonates with CF would have a reduced pH compared to neonates without CF. To test this hypothesis, we measured the pH of nasal ASL because doing so is a non-invasive procedure and because transepithelial electrolyte transport in nasal and tracheal/bronchial epithelia has substantial similarity [11–14]. We studied neonates in an attempt to minimize the potential confounding effects of infection and inflammation. We also measured nasal pH in older children and adults.

2. Methods

All newborns in Iowa undergo a dried blood spot test, to screen for several genetic diseases including CF. An immunoreactive trypsinogen (IRT) ≥ 65 ng/ml is considered a positive screening test for CF [15]. During the period from April 2012 until August 2013, we enrolled neonates with a positive CF screen, older children and adults with CF (ages 3 mos. to 60 yrs.), and healthy volunteers. Children and adults with concomitant nasal or paranasal sinus complaints or history of upper respiratory tract infections in the preceding 3 weeks were excluded from study. All studies were approved by the University of Iowa institutional review board (IRB). Informed consent was obtained from the subjects or their legally authorized representative.

The parents of 31 neonates consented to participate in the study. We excluded one neonate with an IRT 99, sweat Cl^- 7/9 and genotype F508C/3120 + 1G > A. The F508C mutation has been reported either as benign with normal clinical and epithelial physiological studies in two healthy subjects with F508del/F508C mutations [16], or as disease-causing in one subject with typical symptoms of CF and pancreatic insufficiency carrying F508C/unknown mutations [17]. This neonate had a nasal ASL pH of 4.1. Adding this subject to either the CF or the non-CF groups did not change the conclusions.

We used a Sandhill ZepHr PHNS-P (Sandhill Scientific, Highlands Ranch, CO) Mobidium pH probe with an internal reference electrode. Prior to each study, the pH probe was calibrated in buffer solutions of pH 6, 7 and 8 (VWR, West Chester, PA). Voltage was recorded with an Oakton pH6+ meter (Cole-Parmer, Vernon Hills, IL) and corrected to temperature. The probe was positioned 6 cm (adults), 1.5 cm (children) and 1.0 cm (neonates) from the most caudal aspect of the columella (Supplemental Figure S1). The catheter remained in position until the reading was stable for 15 s. All measurements were taken by the same operator. In neonates, the operator was blinded to diagnosis and measurements were obtained within 3 months after birth.

$NaHCO_3$ or NaCl were prepared as 5% solutions and administered intra-nasally at the same time to opposite nostrils using a 250 μl preloaded Accuspray syringe (Becton Dickinson Pharmaceutical Systems, Franklin Lakes, NJ) [18].

3. Statistical analyses

Statistical significance was evaluated by Student's *t* test. For subgroups analysis in Fig. 1C, we used one-way ANOVA with Bonferroni's multiple comparisons test.

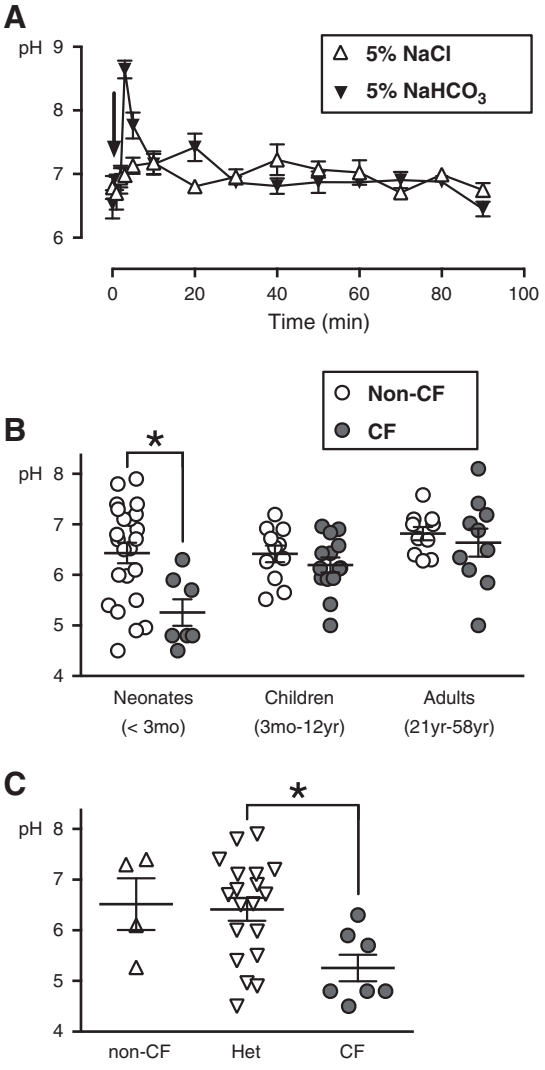

Fig. 1. Nasal ASL pH. **A.** pH of nasal ASL in healthy volunteers (n = 5) before and after aerosol administration of a 5% $NaHCO_3$ or 5% NaCl solutions. Data are mean ± SEM; some error bars are hidden by symbols. **B.** Nasal pH in non-CF (white, n = 23) and CF (dark gray, n = 7) neonates, children (n = 11 non-CF and n = 14 CF), and adults (n = 10 non-CF and n = 10 CF). Data points are values for individuals, bars are means ± SEM. * p <0.01 (Student's t test). **C.** Nasal pH in neonates with no *CFTR* mutations (n = 4), neonates heterozygous for a *CFTR* mutation (n = 19) and neonates with CF (n = 7). * p <0.01 (one-way ANOVA, Bonferroni's multiple comparisons test).

Table 1
Clinical characteristics of neonates.

Subject	Age	Sex	Mutation 1	Mutation 2	Sweat Cl^-	IRT	pH
Non-CF	19 d	F	None	None	10/12	66	5.3
Non-CF	29 d	F	None	None	11/8	178	7.3
Non-CF	20 d	M	None	None	17/14	189	6.1
Non-CF	23 d	M	None	None	13/18	250	7.4
Het	20 d	F	F508del	None	20/22	72	6.5
Het	29 d	M	R553X	None	5/10	81	5.5
Het	22 d	M	F508del	None	11/13	75	6.7
Het	22 d	M	F508del	None	11/12	69	7.9
Het	19 d	M	F508del	None	11/19	72	7.4
Het	33 d	F	F508del	None	6/7	64	5.0
Het	26 d	M	N1303K	None	7/13	70	5.4
Het	13 d	F	G551D	None	7/8	148	4.5
Het	79 d	F	F508del	None	5/5	68	4.9
Het	9 d	M	F508del	None	40/33	65	6.5
Het	27 d	F	F508del	None	13/12	94	6.9
Het	35 d	M	F508del	None	15/11	115	6.8
Het	28 d	F	R553X	None	8/9	88	7.8
Het	34 d	M	F508del	None	10/10	74	7.1
Het	23 d	F	F508del	None	8/13	69	7.2
Het	14 d	F	F508del	None	9/9	81	6.0
Het	22 d	F	F508del	None	5/5	67	6.7
Het	23 d	F	F508del	None	5/6	83	6.0
Het	12 d	F	F508del	None	5/9	67	7.1
CF	13 d	F	F508del	F508del	66/89	231	4.5
CF	13 d	M	F508del	G542X	95/99	87	4.8
CF	9 d	M	G542X	N1303K	91/95	166	4.8
CF	21 d	M	R668C, G576A, D443Y	F508del	41/44	65	4.8
CF	15 d	M	F508del	G551D	75/81	195	6.3
CF	44 d	F	F508del	G542X	69/70	423	5.9
CF	13 d	F	F508del	F508del	32/–	132	5.7

4. Results

As a test of the pH assay, we applied $NaHCO_3$ as an aerosol spray to the nasal surface and measured pH in 5 healthy adults. Administering 5% $NaHCO_3$ immediately and transiently alkalinized ASL pH, whereas 5% NaCl had little effect (Fig. 1A). Additional experiments demonstrated concordance of measurements between right and left nostrils, and reproducibility of the assay (Supplemental Figures S2, and S3).

To recruit neonates, we evaluated babies with a positive screening test for CF. Newborns in Iowa are screened by measuring blood IRT; those with a positive test are referred for additional testing. A fraction of those infants will receive a diagnosis of CF. Thus, this population yielded babies with CF as well as non-CF babies who served as age-matched controls. We studied infants before results of their genetic tests or sweat tests were known. Thus, operators were unaware of the diagnosis.

We measured nasal pH 24 ± 2 days (range, 9–79 days) after birth. Table 1 shows the genotypes and demographics of 7 babies with CF and 23 without CF. In CF neonates, the nasal pH of 5.2 ± 0.3 was more acidic than the pH of 6.4 ± 0.2 in non-CF neonates (Fig. 1B).

Because a previous study of older subjects did not detect a CF:non-CF difference [11], we measured nasal pH in people with CF beyond the neonatal period (Table 2). In contrast to our findings in neonates, the nasal pH of CF children and adults was similar to values measured in people without CF (Fig. 1B). Our cohort of children and adults with CF included subjects with mutations known to exhibit partial CFTR activity (Table 2). Excluding these subjects from the analysis did not affect the results.

In a separate analysis, we tested whether neonates heterozygous for a CF-associated mutation had a reduced nasal pH and found that their pH (pH = 6.4 ± 0.2, $n = 19$) did not statistically differ from that in neonates with no *CFTR* mutations (pH = 6.5 ± 0.5, $n = 4$). However, our ability to detect a difference between heterozygotes and neonates with no *CFTR* mutations was limited by the small number of babies. The nasal pH of heterozygous neonates was higher than that in neonates with two *CFTR* mutations (Fig. 1C).

5. Discussion

Data from this small, single center pilot study suggest that neonates with CF have an acidic nasal ASL compared to babies without CF. Although infection, inflammation and airway wall remodeling begin soon after birth, obtaining measurements in neonates should minimize the effect of those disease consequences on ASL pH. We also found that the CF:non-CF difference disappeared in older individuals.

Our results emphasize a distinction between airways not affected by infection, inflammation and remodeling and airways studied later in the course of disease. In cultured human and porcine airway epithelia, ASL pH was lower in CF

Table 2
Clinical characteristics of children (age 3 mos. to 12 yrs.) and adults (age 21 yrs. to 58 yrs.) with CF.

Age	Sex	Mutation 1	Mutation 2	Sweat Cl^-	FEV1	%FEV1	Col	pH
3 m	F	F508del	p.1336 K	85/81	–	–	N	6.13
4 m	M	F508del	F508del	85/89	–	–	N	6.43
7 m	F	F508del	G542X	94/88	–	–	S	6.84
9 m	F	F508del	1973_1985del13insAGAAA	102/97	–	–	N	6.12
2 y	F	F508del	F508del	99/115	–	–	S, P	5.94
2 y	M	F508del	F508del	95/94	–	–	S, P	6.58
3 y	F	F508del	R117H	24/29	–	–	P	6.43 [a]
3 y	M	F508del	F508del	89/83	–	–	S, P	5.94
3 y	M	F508del	F508del	67/56	–	–	S	6.90
6 y	F	F508del	S549N	78/82	–	–	S, P	6.96
6 y	F	G542X	1717G > A	116/118	–	–	S, P	5.92
11 y	M	F508del	1154insTC	130/131	–	–	S, P	5.42
11 y	M	F508del	F508del	101/103	–	–	S, P	6.14
12 y	F	R64Q, R297Q	Y109N	39/49	–	–	S	5.00 [a]
21 y	F	F508del	F508del	98/107	3.01	100	S, P	6.88
23 y	F	F508del	F508del	70/65	1.86	56	S, P	7.20
28 y	M	F508del	F508del	81/94	1.15	28	S, P	7.02
30 y	M	F508del	F508del	74/53	2.64	94	P	6.10
30 y	F	F508del	F508del	70/83	2.88	83	S, P	7.42
40 y	M	F508del	F508del	80/113	3.84	82	P	6.49
46 y	M	F508del	R347H	73/79	1.55	36	P	6.34
47 y	F	D1152H	F508del	–	2.76	95	N	8.10 [a]
56 y	M	F508del	G551D	139/141	2.14	61	P	5.00
58 y	F	F508del	3849 + 10	46/44	2.76	76	P	5.84 [a]

col = colonization, S = *S. aureus*, P = *P. aeruginosa*, N = no bacteria detected
[a] mutations with known partial CFTR activity

than non-CF [5,9]; the culture environment for these airways was identical for both genotypes. For CF pigs studied the day they are born and before infection and inflammation *in vivo* and *ex vivo* assays showed that CF pigs have an abnormally acidic pH [9]. A study of submucosal gland secretions from nasal tissue of people with CF undergoing sinus surgery also revealed an acidic ASL pH [8]. These findings contrast with two other studies of airways likely to be involved by secondary consequences of the disease. The pH of submucosal gland secretions from CF airways removed at the time of lung transplantation did not differ from non-CF [19]. In addition, a study of adults with CF and pediatric patients with CF (3–16 years old) observed no CF:non-CF difference in ASL pH [11]. Thus, our data in neonates and older people are consistent with earlier studies, and together they suggest that infection, inflammation, airway remodeling and/or other factors minimize CF:non-CF differences in ASL pH.

Our data do not reveal the mechanisms that might eliminate the pH differences with time. Developmental changes in the mechanisms that control ASL pH are possible. However, earlier studies in non-CF people showed that inflammation increased ASL pH [20,21]. Furthermore, measuring exhaled breath condensate pH in CF patients showed no difference from control group when the confounding effect of exhaled CO_2 on pH was taken into account [22–24]. We speculate that ASL pH increased in CF airways because inflammation altered pH regulatory mechanisms, whereas in the absence of inflammation, non-CF ASL pH was unaltered.

Additional larger studies will be required to validate these findings. If they do, the results have important implications. First, they suggest that reduced ASL pH may be a key abnormality that initiates airway disease. Second, it is possible that ASL pH becomes a less important pathogenic factor as disease progresses. Perhaps at that time, other factors become more important in fueling disease progression. Third, normalizing ASL pH in infants may be a therapeutic strategy and measurements of ASL pH might report on the success of therapeutic interventions.

Conflict of interest statement

None of the authors have any commercial or other associations that would pose a conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jcf.2013.12.006.

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