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Short Communication

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

Mahmoud H. Abou Alaiwa [a], Alison M. Beer [a], Alejandro A. Pezzulo [a], Janice L. Launspach [a], Rebecca A. Horan [a], David A. Stoltz [a], Timothy D. Starner [b], Michael J. Welsh [a,c,d,*], Joseph Zabner [a,**]

[a] Department of Internal Medicine, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA 52242, USA
[b] Department of Pediatrics, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA 52242, USA
[c] Howard Hughes Medical Institute, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA 52242, USA
[d] Department of Molecular Physiology and Biophysics, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA 52242, USA

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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

* Correspondence to: M. J. Welsh, HHMI/University of Iowa Department of Medicine, 500 EMRB, Iowa City, IA 52242, USA. Tel.: +1 319 335 7619; fax: +1 319 335 7623.
** Correspondence to: J. Zabner, University of Iowa Department of Medicine, 440 EMRB, Iowa City, IA 52242, USA. Tel.: +1 319 356 4419; fax: +1 319 356 8101.
E-mail addresses: michael-welsh@uiowa.edu (M.J. Welsh), joseph-zabner@uiowa.edu (J. Zabner).

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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.

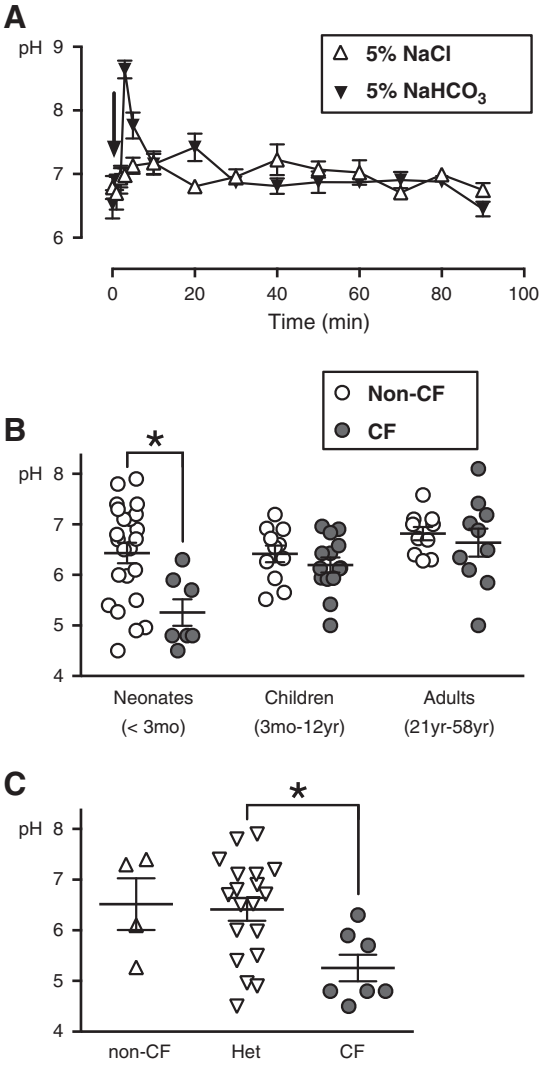

Fig. 1. Nasal ASL pH. **A.** pH of nasal ASL in healthy volunteers (n = 5) before and after aerosol administration of a 5% $NaHCO_3$ or 5% NaCl solutions. Data are mean ± SEM; some error bars are hidden by symbols. **B.** Nasal pH in non-CF (white, n = 23) and CF (dark gray, n = 7) neonates, children (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).