Ivacaftor-induced sweat chloride reductions correlate with increases in airway surface liquid pH in cystic fibrosis

Mahmoud H. Abou Alaiwa, … , Edward F. McKone, Michael J. Welsh


BACKGROUND. Disruption of cystic fibrosis transmembrane conductance regulator (CFTR) anion channel function causes cystic fibrosis (CF), and lung disease produces most of the mortality. Loss of CFTR-mediated HCO₃⁻ secretion reduces the pH of airway surface liquid (ASL) in vitro and in neonatal humans and pigs in vivo. However, we previously found that, in older children and adults, ASL pH does not differ between CF and non-CF. Here, we tested whether the pH of CF ASL increases with time after birth. Finding that it did suggested that adaptations by CF airways increase ASL pH. This conjecture predicted that increasing CFTR activity in CF airways would further increase ASL pH and also that increasing CFTR activity would correlate with increases in ASL pH.

METHODS. To test for longitudinal changes, we measured ASL pH in newborns and then at 3-month intervals. We also studied people with CF (bearing G551D or R117H mutations), in whom we could acutely stimulate CFTR activity with ivacaftor. To gauge changes in CFTR activity, we measured changes in sweat Cl⁻ concentration immediately before and 48 hours after starting ivacaftor.

RESULTS. Compared with that in the newborn period, ASL pH […]

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Ivacaftor-induced sweat chloride reductions correlate with increases in airway surface liquid pH in cystic fibrosis

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Introduction

Cystic fibrosis (CF) is caused by mutations in the gene encoding CF transmembrane conductance regulator (CFTR), and lung disease is the major source of morbidity and mortality (1–3). CFTR is an apical membrane anion channel that provides a pathway for secretion of Cl− and HCO3− into the airway surface liquid (ASL) and submucosal gland lumen (4, 5).

The loss of CFTR-mediated HCO3− secretion in CF predicts that the HCO3− concentration and pH of ASL and submucosal gland secretions will be reduced. Consistent with this prediction, ASL in differentiated cultures of human CF airway epithelia has a lower pH than non-CF epithelia in most measurements (6–9). ASL was also abnormally acidic in airway epithelia cultured from CF pigs (7, 10–13), which develop airway disease similar to that in humans (14, 15). Submucosal gland secretions from human CF epithelia...
were also abnormally acidic (16). In addition, in vivo and ex vivo studies of newborn CF pigs revealed an abnormally acidic ASL pH (7, 10). In CF rats, in vivo ASL pH was abnormally acidic at 1–6 months after birth (17). The acidic pH and reduced HCO$_3^-$ concentration disrupt respiratory host defense by impairing mucociliary transport and antimicrobial activity (10, 11, 18–20).

In an earlier pilot study, we measured the pH of nasal ASL in neonates identified by newborn screening for CF, but before it was known whether or not they had CF. Compared with non-CF neonates, CF neonates (<1 month old) had a lower ASL pH (21). Yet in older children (median age, 3 years) and adults with CF, we found that ASL pH did not differ from that of people without CF (21). An earlier study also found no CF/non-CF difference in older children (median age, 12 years) or adults (22). A recent study of children (median age, 4.2 years) also reported no difference between genotypes (9). However, how ASL pH changes in an individual after the neonatal period is unknown.

Our in vivo results suggest that, in CF, ASL pH may increase with time to reach the pH of non-CF ASL. That could occur if CFTR-independent mechanisms increase ASL pH in CF. From that scenario, we made two predictions. (a) If we could precisely reproduce CF-induced secondary changes in non-CF airways, then CFTR-independent mechanisms occurring in addition to CFTR HCO$_3^-$ secretion would further increase ASL pH. How we might do this is unclear. (b) If we could acutely restore CFTR activity to CF airways, then CFTR activity occurring in addition to CFTR-independent changes would raise pH. We hypothesized that, in adults with CF, increasing CFTR activity would increase ASL pH. We also hypothesized that a greater increase in CFTR activity would produce a greater increase in ASL pH.

To test the second prediction, we studied people with CF who carry either a G551D or R117H mutation and increased CFTR activity with ivacaftor. We had an opportunity to perform this study when ivacaftor was approved in Ireland as a treatment for patients carrying a G551D or R117H mutation. Ivacaftor immediately increases the open-state probability of CFTR-G551D and CFTR-R117H channels, reduces sweat [Cl$^-$], and increases pulmonary function measured as the percentage predicted forced expiratory volume in 1 second (FEV$_1$%) (23–26). In addition, when at least one CFTR allele has a G551D mutation, ivacaftor increases ASL pH in cultured CF airway epithelia (27) and pH in the small intestinal lumen of people with CF (28). Our hypothesis was that, as CFTR activity increases, ASL pH would increase. To gauge ivacaftor-induced increases in CFTR activity, we measured the change in sweat [Cl$^-$]. Changes in sweat [Cl$^-$] are currently the most reliable and accessible means of assessing changes in CFTR activity in vivo. We also measured changes in FEV$_1$%. We studied people immediately before and 48 hours after beginning the drug; ivacaftor reaches steady-state concentrations in 2–3 days (29). This approach minimizes secondary consequences of restoring CFTR function and reduces the influence of changes in disease severity.

Results

CF ASL pH is abnormally acidic at birth and increases with time. In an earlier pilot study, we measured ASL pH in neonates who screened as positive for immunoreactive trypsinogen before the sweat [Cl$^-$] or genetic results were known (21). Thus, we were blinded to genotype. Since then, we have studied an additional 5 neonates. Compared with infants without CF, a combined group of 11 CF neonates (Table 1) had a lower ASL pH (Figure 1A).

To test whether ASL pH increases with time, we studied the babies with CF after the initial analysis. Compared with the initial measurement in newborn babies with CF, by 6 months of age, ASL pH was increased (Figure 1B). All the babies had CF pathogens present in cultures and/or neutrophilic inflammation in bronchoalveolar lavage fluid, consistent with previous reports (30–32).

These data suggest that after the neonatal period, CFTR-independent mechanisms increase ASL pH in CF airways. The cause is unknown. We speculate that airway inflammation, infection, and remodeling might be responsible. Consistent with that speculation, cytokines that are elevated in CF airways, such as IL-17, IL-1β, IL-4, IL-13, and IFN-γ, raise ASL pH in non-CF epithelia (33–36), and IL-1β has been reported to increase paracellular permeability (37). Moreover, viral infections, with their associated inflammation, can increase ASL pH in vivo (38). However, there are also reports that ASL pH is decreased in exhaled breath condensate of people with asthma and CF (39, 40). Because ASL pH did not differ among neonates, older children, and adults without CF, developmental changes seem less likely to be responsible (21). However, we cannot exclude the possibility that developmental differences in CF and non-CF airways are responsible. If ASL pH increases in CF airways after the neonatal period, then acutely adding CFTR activity in addition to those changes should further increase ASL pH. We tested that prediction using ivacaftor.
Increases in ASL pH and FEV₁% correlate with reductions in sweat [Cl⁻]. We studied people with CF who carried a G551D or R117H mutation and made measurements before and 48 hours after beginning ivacaftor. We previously reported sweat [Cl⁻] and FEV₁% for people with at least one copy of a G551D mutation (41–43). Here, we performed a similar study in people with CF carrying at least one R117H mutation. We combined the data for sweat [Cl⁻] and FEV₁% for people with either mutation (Table 2). The sweat [Cl⁻] fell, and FEV₁% increased (Figure 2, A and B). We also measured the pH of nasal ASL in both groups. In contrast to our prediction, ASL pH did not change significantly (Figure 2C). However, given the variance of ASL pH measurements, we were markedly underpowered to detect a difference of 0.2 pH units or greater. Ivacaftor will increase CFTR activity to variable extents in different individuals. Therefore, we predicted that a greater change in sweat [Cl⁻] would be associated with a greater change in FEV₁%. That was the case (Figure 3A). We also predicted that even though ivacaftor did not significantly increase average ASL pH, there would be a correlation between the magnitude of change in CFTR activity (measured as sweat [Cl⁻]) and the change in ASL pH. We found that, when ivacaftor produced a greater reduction in sweat [Cl⁻], there was a greater increase in ASL pH (Figure 3B). There was also a trend (P = 0.13) for a positive correlation between increases in ASL pH and FEV₁% (Figure 3C). These results suggest that, as CFTR activity increases in CF airways, ASL pH increases.

Discussion

This study had several advantages. First, by studying newborns, we could compare neonates with and without CF before airways developed the marked inflammation and infection that characterize CF. Second, the study population of people with ivacaftor-responsive CFTR mutations allowed us to increase CFTR activity in addition to chronic CF-associated changes in the airways. Third, the design of the study took advantage of the immediate effect of ivacaftor on CFTR (23) and the near steady-state concentration achieved within 2 days of beginning administration (29). That allowed us to make measurements at time 0 and 48 hours and thereby diminish any effect of changes in an individual’s disease severity, metabolism, or environmental exposures. That short interval likely also minimizes secondary consequences of partially restoring CFTR activity. The ability to minimize confounding factors may explain why we found a correlation between an individual’s reduction in sweat [Cl⁻] and an increase in FEV₁%, whereas previous studies had only found a correlation between changes in sweat [Cl⁻] and FEV₁% at the population level (44, 45). Fourth, in contrast to comparing groups of people with and without CF, individuals served as their own controls. The chance to study a group of people who initiated treatment at the same time at one location provided a unique opportunity to address this question. Fifth, these were in vivo measurements rather than assays in culture.

Table 1. Clinical characteristics, throat cultures, and bronchoscopy results of neonates with CF

<table>
<thead>
<tr>
<th>Genotype</th>
<th>IRT (ng/ml)</th>
<th>Sweat [Cl⁻] (mEq/l)</th>
<th>ASL pH</th>
<th>Cultures</th>
<th>BALF</th>
</tr>
</thead>
<tbody>
<tr>
<td>F508del/G551D</td>
<td>195</td>
<td>78</td>
<td>6.6</td>
<td>3 mo and 15 mo, <em>Pseudomonas aeruginosa</em> and <em>Staphylococcus aureus</em></td>
<td>15 mo, 20% PMNs</td>
</tr>
<tr>
<td>F508del/G542X</td>
<td>87</td>
<td>97</td>
<td>4.8</td>
<td>6 mo, <em>Pseudomonas aeruginosa</em></td>
<td>12 mo, 50% PMNs</td>
</tr>
<tr>
<td>F508del/F508del</td>
<td>231</td>
<td>77</td>
<td>4.5</td>
<td>Neg</td>
<td>18 mo, 50% PMNs</td>
</tr>
<tr>
<td>G542X/N1303K</td>
<td>166</td>
<td>93</td>
<td>4.8</td>
<td>12 mo, <em>Streptococcus</em></td>
<td>12 mo, 15% PMNs</td>
</tr>
<tr>
<td>F508del/G542X</td>
<td>423</td>
<td>70</td>
<td>5.9</td>
<td>3 mo and 6 mo, <em>Pseudomonas aeruginosa</em> and 15 mo, <em>Staphylococcus aureus</em></td>
<td>15 mo, 53% PMNs</td>
</tr>
<tr>
<td>F508del/F508del</td>
<td>132</td>
<td>90</td>
<td>5.7</td>
<td>3 mo, <em>Haemophilus</em></td>
<td>Not done</td>
</tr>
<tr>
<td>F508del/N1303K</td>
<td>202</td>
<td>75</td>
<td>5.2</td>
<td>3 mo, <em>Haemophilus</em>; 6 mo, <em>Moraxella</em></td>
<td>6 mo, 4% PMNs</td>
</tr>
<tr>
<td>F508del/F508del</td>
<td>163</td>
<td>95</td>
<td>6.9</td>
<td>Birth, 3 mo, 6 mo, 9 mo, 12 mo, and 18 mo, <em>Staphylococcus aureus</em></td>
<td>9 mo, 12% PMNs</td>
</tr>
<tr>
<td>F508del/F508del</td>
<td>122</td>
<td>106</td>
<td>4.6</td>
<td>Birth and 6 mo, <em>Staphylococcus aureus</em></td>
<td>6 mo, 3% PMNs</td>
</tr>
<tr>
<td>F508del/1998+1G &gt; A</td>
<td>93</td>
<td>104</td>
<td>6.2</td>
<td>Neg</td>
<td>6 mo, 13% PMNs</td>
</tr>
<tr>
<td>F508del/F508del</td>
<td>290</td>
<td>89</td>
<td>6.7</td>
<td>Birth, <em>Stenotrophomonas</em>; 6 mo, <em>Staphylococcus aureus</em></td>
<td>Not done</td>
</tr>
</tbody>
</table>

IRT, immunoreactive trypsinogen; PMNs, polymorphonuclear cells; BALF, Bronchoalveolar lavage fluid. *Data for these infants can be found in ref. 21. There were no previous longitudinal data for any of these babies. Data for sweat [Cl⁻] are average of 2 measurements.
This study also has limitations. We measured ASL pH on nasal rather than intrapulmonary epithelia. However, the pH of nasal ASL correlates closely with pH of lower airway ASL (22). In addition, it would be important to measure pH in the lumen of submucosal glands where much of the mucus and liquid is generated, but this is not currently possible in vivo (5, 16). Another limitation is that ASL pH values showed substantial variability; how much technical and biological factors contribute to the variation is uncertain.

ASL pH is controlled by CFTR-mediated HCO₃⁻ secretion and by CFTR-independent processes. At birth, loss of CFTR reduces ASL pH. At later time points, CFTR-independent adaptations increase pH in CF ASL, and CF and non-CF adults and older children have a similar ASL pH, despite the loss of CFTR-mediated HCO₃⁻ secretion. Our longitudinal data and the response to ivacaftor support these conclusions but do not identify the non-CFTR mechanisms that increase ASL pH in CF. We speculate that the pH of non-CF ASL would be higher if non-CF airways had infection, inflammation, and remodeling.

Table 2. Baseline clinical characteristics of study subjects

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Age</th>
<th>Sex</th>
<th>% Predicted FEV₁ (L)</th>
<th>Sweat [Cl⁻] (mEq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G551D/F508del</td>
<td>23</td>
<td>F</td>
<td>79 (2.8 L)</td>
<td>99.9</td>
</tr>
<tr>
<td>G551D/F508del</td>
<td>25</td>
<td>F</td>
<td>72 (2.42 L)</td>
<td>102.6</td>
</tr>
<tr>
<td>G551D/F508del</td>
<td>23</td>
<td>F</td>
<td>79 (2.46 L)</td>
<td>90.6</td>
</tr>
<tr>
<td>G551D/F508del</td>
<td>30</td>
<td>F</td>
<td>40 (1.11 L)</td>
<td>65.7</td>
</tr>
<tr>
<td>G551D/F508del</td>
<td>27</td>
<td>F</td>
<td>51 (1.49 L)</td>
<td>91.5</td>
</tr>
<tr>
<td>G551D/3659delC</td>
<td>22</td>
<td>F</td>
<td>39 (1.25 L)</td>
<td>109</td>
</tr>
<tr>
<td>G551D/F508del</td>
<td>33</td>
<td>F</td>
<td>88 (2.54 L)</td>
<td>105</td>
</tr>
<tr>
<td>G551D/p67L</td>
<td>57</td>
<td>F</td>
<td>34 (0.82 L)</td>
<td>80.9</td>
</tr>
<tr>
<td>G551D/F508del</td>
<td>35</td>
<td>F</td>
<td>77 (2.16 L)</td>
<td>98.6</td>
</tr>
<tr>
<td>G551D/G551D</td>
<td>29</td>
<td>M</td>
<td>101 (3.99 L)</td>
<td>110.3</td>
</tr>
<tr>
<td>G551D/F508del</td>
<td>30</td>
<td>M</td>
<td>39 (1.6 L)</td>
<td>108.2</td>
</tr>
<tr>
<td>G551D/R117H</td>
<td>33</td>
<td>M</td>
<td>72 (2.76 L)</td>
<td>61.9</td>
</tr>
<tr>
<td>R117H/F508del</td>
<td>45</td>
<td>M</td>
<td>108 (3.80 L)</td>
<td>98</td>
</tr>
<tr>
<td>R117H/F508del</td>
<td>40</td>
<td>M</td>
<td>96 (3.75 L)</td>
<td>81</td>
</tr>
<tr>
<td>R117H/F508del</td>
<td>41</td>
<td>F</td>
<td>87 (2.44 L)</td>
<td>61</td>
</tr>
<tr>
<td>R117H/F508del</td>
<td>34</td>
<td>F</td>
<td>62 (1.81 L)</td>
<td>71</td>
</tr>
<tr>
<td>R117H/F508del</td>
<td>40</td>
<td>M</td>
<td>75 (3.05 L)</td>
<td>78</td>
</tr>
<tr>
<td>R117H/F508del</td>
<td>52</td>
<td>M</td>
<td>69 (2.39 L)</td>
<td>90</td>
</tr>
<tr>
<td>R117H/M156R</td>
<td>42</td>
<td>F</td>
<td>43 (1.37 L)</td>
<td>61</td>
</tr>
<tr>
<td>R117H/M156R</td>
<td>40</td>
<td>M</td>
<td>35 (1.49 L)</td>
<td>66</td>
</tr>
<tr>
<td>R117H/2622+1G→A</td>
<td>25</td>
<td>M</td>
<td>75 (3.41 L)</td>
<td>89</td>
</tr>
<tr>
<td>R117H/F508del</td>
<td>46</td>
<td>F</td>
<td>62 (1.90 L)</td>
<td>80</td>
</tr>
</tbody>
</table>
identical to that in CF airways. Conversely, we speculate that the pH of ASL in CF adults might be lower in the absence of CFTR-independent adaptations.

These results may also have implications for respiratory host defense. Previous studies showed that there may not be a single optimal value for ASL pH; as pH and HCO$_3^-$ concentration increase, MCT and antimicrobial activity increase (10–12, 46). Therefore, increasing ASL pH may be of therapeutic benefit. Thus, a better understanding of the mechanisms responsible for a CFTR-independent increase in ASL pH might be exploited to enhance airway defense in CF and perhaps other airway diseases.

Methods

Study participants. From April 2012 until June 2016, in Iowa, we enrolled 56 newborns who had a screening immunoreactive trypsinogen $\geq 65$ ng/ml. We measured nasal ASL pH at their first clinic visit (age varied from a few days after birth to $<1$ month), before results of the sweat [Cl–] or genetic test results were known. For many of the participants, we were able to repeat the measurement of nasal ASL pH at 3-month intervals over the course of 18 months. Table 1 shows the genotypes, sweat [Cl–], and initial ASL pH. The table also shows evidence of the development of CF airway disease based on throat swab cultures and fiber optic bronchoscopic examination of the airways with collection of bronchoalveolar lavage.

We enrolled adult subjects with CF who had at least one $G551D$ or $R117H$ allele in two prospective observational studies at a single CF center, the National Referral Centre for Adult Cystic Fibrosis. Inclusion criteria included an age $\geq 18$ years (both male and female subjects were enrolled), a clinical diagnosis of CF and a CFTR-null mutation, and an FEV$_1$ $\geq 40$% predicted. We did not detect an effect of sex or genotype on the results. *$P < 0.001$ by Wilcoxon matched-pairs signed-rank test.
a G551D-CFTR or R117H-CFTR mutation on at least 1 allele, clinical stability with no significant changes in health status within 14 days prior to visit 1, and a negative serum pregnancy test at screening for females of childbearing potential. Exclusion criteria included participation in the VX-770 Extended Access Program or use of ivacaftor within 6 months prior to visit 1; an acute upper or lower respiratory infection, pulmonary exacerbation, or changes in therapy (including antibiotics) for pulmonary disease within 4 weeks of visit 1; history of solid-organ transplantation; ongoing pregnancy or breastfeeding; ongoing participation in another therapeutic clinical study or prior participation in an investigational drug study within 30 days before screening; use of any inhibitors or inducers of cytochrome P450 3A4; any non–CF-related illness within 2 weeks before visit 1; or abnormal renal or liver function at screening. Standard therapy for CF was allowed to continue, except for inhaled bronchodilators that were held the morning of visits. Table 2 summarizes the clinical characteristics of participants. Measurements of sweat [Cl–], spirometry, and ASL pH were obtained at baseline and 2 days after starting ivacaftor. Values of sweat [Cl–] and FEV1% for participants with a G551D mutation have been reported previously (41–43).

Sweat [Cl–]. Sweat was collected with the Macroduct collection system (Wescor), and sweat [Cl–] was measured using routine laboratory techniques.

Spirometry. Spirometric measurements were obtained in accordance with the American Thoracic Society Standards. The FEV1 predicted values are based on the European Community for Coal and Steel 1993 values for adults.

ASL pH. We used a Sandhill ZepHr PHNS-P (Sandhill Scientific) Mobidium pH probe with an internal reference electrode to measure ASL pH in the nose. Prior to each study, the pH probe was calibrated in buffer solutions of pH 6, 7, and 8 (VWR). Voltage was recorded with an Oakton pH6 + meter (Cole-Parmer) and corrected to temperature. The probe was positioned 1 cm (neonates) or 6 cm (adults) from the most caudal aspect of the columella. The catheter remained in position until the reading was stable for 15 seconds. All measurements were taken by the same operator. One person in the ivacaftor study declined measurement of ASL pH.

Statistics. Data are presented as values for individual participants with mean ± SD indicated by bars. For statistical analysis, we used a Mann-Whitney test for unpaired comparisons and reported significance with a 2-tailed P value. For paired comparisons, we used Wilcoxon matched-pairs signed-rank test and reported significance with a 2-tailed P value. In Figure 1B, at 6 months, we had ASL pH measurements from 10 of the 11 neonates. Therefore, we used this time point to compare to measurements at birth. In Figure 3, we tested correlations with a nonparametric Spearman correlation coefficient. Differences were considered statistically significant at P < 0.05.

Study approval. All participants (and their guardians, as applicable) provided written informed consent. The studies were approved by the University of Iowa Institutional Review Board and the Research Ethics Committee of St. Vincent’s University Hospital.

Author contributions
MHAA, JZ, DAS, PKS, EFM, and MJW led the study design. MHAA, JLL, BG, SC, JZ, DAS, PKS, EFM, and MJW contributed to data collection and data analyses. MHAA, JZ, DAS, PKS, EFM, and MJW interpreted the data. MHAA and MJW drafted the manuscript. MHAA, JLL, BG, SC, JZ, DAS, PKS, EFM, and MJW contributed to revisions.

Acknowledgments
We thank the participants who made this study possible. We are grateful to Beth Dowd and Lauri Ramsey for help with study participants. This study was supported by an investigator-initiated award from Vertex Inc. (to PKS and EFM), the Cystic Fibrosis Foundation (SINGH17A0 and SINGH15R0), and the NIH (P30DK089507). MHAA is supported in part by the NIH (1K08HL135433). JZ is supported in part by the NIH (HL091842). DAS is supported in part by the NIH (HL136813). PKS is supported in part by the NIH (K24HL102246) and the Burroughs Wellcome Fund. MJW is an Investigator of the Howard Hughes Medical Institute.

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