Safety of an Alkalinizing Buffer Designed for Inhaled Medications in Humans

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BACKGROUND: Airway acidification plays a role in disorders of the pulmonary tract. We hypothesized that the inhalation of alkalinized glycine buffer would measurably alkalinize the airways without compromising lung function or causing adverse events. We evaluated the safety of an inhaled alkaline glycine buffer in both healthy subjects and in subjects with stable obstructive airway disease. METHODS: This work includes 2 open-label safety studies. The healthy controls were part of a phase 1 safety study of multiple inhalations of low-dose alkaline glycine buffer; nebulized saline was used as a comparator in 8 of the healthy controls. Subsequently, a phase 2 study in subjects with stable obstructive airway disease was completed using a single nebulized higher-dose strategy of the alkaline inhalation. We studied 20 non-smoking adults (10 healthy controls and 10 subjects with obstructive airway disease), both at baseline and after inhalation of alkaline buffer. We used spirometry and vital signs as markers of clinical safety. We used changes in fraction of exhaled nitric oxide (NO) and exhaled breath condensate (EBC) pH as surrogate markers of airway pH modification. RESULTS: Alkaline glycine inhalation was tolerated by all subjects in both studies, with no adverse effects on spirometric parameters or vital signs. Airway alkalinization was confirmed by a median increase in EBC pH of 0.235 pH units (IQR 0.56–0.03, P = .03) in subjects after inhalation of the higher-dose alkaline buffer (2.5 mL of 120 mmol/L glycine). CONCLUSIONS: Alkalinization of airway lining fluid is accomplished with inhalation of alkaline glycine buffer and causes no adverse effects on pulmonary function or vital signs. Key words: airway acidification; glycine; buffer; COPD; exhaled nitric oxide; NO; exhaled breath condensate; airway pH. [Respir Care 2013;58(7):1226–1232. © 2013 Daedalus Enterprises]

Introduction

The regulation of airway pH plays a role in the pathogenesis of obstructive lung diseases. Airway acidification, partly supported by National Institute of Nursing Research grant K99/R00 NR012016. This research was funded in part by the National Institute of Health and the University of Virginia Philip Morris Tobacco Research Fund.

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Mr Davis is affiliated with the Adult Health and Nursing System, Virginia Commonwealth University, Richmond, Virginia. Mr Dwyer, Mrs Combs, Dr Vehse, and Dr Hunt are affiliated with the Division of Pediatric Respiratory Medicine; Dr Paget-Brown is affiliated with the Division of Neonatology; and Dr Pajewski is affiliated with the Department of Anesthesiology, University of Virginia, Charlottesville, Virginia. At the time of this study Mr Walsh was affiliated with the Children’s Medical Center of Dallas, Dallas, Texas; he is now with Boston Children’s Hospital, Boston, Massachusetts.

Dr Hunt has disclosed a relationship with Respiratory Research Inc, Austin, Texas, which manufactures exhaled breath condensate collection equipment. Dr Hunt and the University of Virginia have intellectual property interest in airway pH diagnosis and therapy. Mr Davis was caused by both intrinsic and extrinsic factors, is associated with neutrophilic and eosinophilic inflammation, bronchospasm, bronchial hyper-reactivity, ciliary dysfunction, ep-
ithelial dysfunction, augmented oxidative damage, abnormal fluid transport, inhibition of transport of cationic drugs such as albuterol, and alteration of cellular death pathways, including inhibition of apoptosis.\textsuperscript{3} Knowledge of the role of airway pH in pulmonary health, along with the development of devices and techniques to measure it, has created interest in treatment of airway pH disturbances. Improved ability to treat or to potentially reverse acidic airway pathology by means of therapeutic alteration of airway pH could have an impact in respiratory medicine. The ability to normalize airway pH via inhalation\textsuperscript{2} may allow introduction of new pulmonary therapeutics.

Airway lining fluid (ALF) acidity can be qualitatively determined noninvasively via the collection of exhaled breath condensate (EBC) and the measurement of its pH.\textsuperscript{3} Assays for the measurement of EBC pH have been developed for patients of all ages and sizes, including those receiving mechanical ventilation.\textsuperscript{4-7} EBC pH normally lies within a mildly alkaline range of 7.5–8.2.\textsuperscript{7-19} EBC has a minimal buffer capacity, which allows EBC to assess the presence of volatile acids in ALF, as indicated by a change in its pH.\textsuperscript{20,21} Although a normal EBC pH does not exclude airway acidity at some level, a low EBC pH value is highly specific for acidity somewhere within the airway. Using EBC methods, several studies have shown that patients with COPD,\textsuperscript{13} asthma,\textsuperscript{7,9} bronchiectasis,\textsuperscript{11} cystic fibrosis,\textsuperscript{9} and chronic cough\textsuperscript{15} have airway acidification. We incorporated EBC into this study as a noninvasive safety measure to assess for the possibility of excessive alkalization from our intervention.

Airway pH also affects fraction of exhaled nitric oxide (FeNO) levels by simple chemistry. As the pH of ALF decreases, commonly present nitrite becomes protonated to nitrous acid, which decomposes to nitric oxide,\textsuperscript{22} which is then in part exhaled. F\textsubscript{eNO} analyzers may be used qualitatively to longitudinally assess the alkalizing effects of alkaline inhalation therapy through monitoring decreases in F\textsubscript{eNO} levels.\textsuperscript{2,23} The first aim of the present study was to evaluate the safety of an inhaled aerosol of alkaline glycine buffer in healthy controls; we also investigated the potential of this inhaled, aerosolized buffer to alkalize the ALF pH in both healthy subjects and those with stable obstructive airway disease.

Methods

Protocol

Two open-label safety studies were approved by the University of Virginia institutional review board under an Investigational New Drug Application from the United States Food and Drug Administration. Approval and initiation of the second study by the University of Virginia institutional review board was dependent upon successful completion of the first study and results indicating safety of the intervention. We obtained informed consent from all subjects for both studies. The first study recruited 10 healthy volunteers via direct approach from the study team. The study was performed in the General Clinical Research Center. No reimbursement was provided. The second study recruited 10 subjects with stable obstructive lung disease, via referral from their allergist, and was performed in the Allergy/Asthma/Immunology Clinic. Subjects were reimbursed $100 for participation. An independent medical safety monitor reviewed the results for each subject daily during the studies. Both studies were executed between 10:00 AM and 2:00 PM Eastern Standard Time.

For the first study (study 1) we acquired (in the following order) baseline F\textsubscript{eNO} levels, EBC samples, spirometry (FEV\textsubscript{1}, FVC, FEV\textsubscript{1}/FVC, and forced expiratory flow during the middle half of the FVC maneuver [FEF\textsubscript{25-75}%]), and vital signs (heart rate, breathing frequency, oxygen saturation, and breath sounds) from the subjects. The order in which these measurements were obtained was kept consistent throughout all points of the study, with the exception of EBC, which was collected only before the first inhalation and immediately after the third inhalation. All of the subjects were within healthy limits for spirometry (baseline > 80% of predicted for all observed values) and vital signs, and had not ingested anything except water for 8 hours prior to the study. Exclusion criteria included a > 5 pack-year smoking history, current pregnancy (all female subjects were either abstinent, post-menopausal, or practicing adequate contraception), a history of pulmonary disease (verified through verbal medical history), or acute illness (verified by clinical history or reported by the subject) within 5 days of study.

The study 1 treatment preparation was an isotonic solution of a sodium chloride diluent mixed with glycine to
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Fig. 1. Timeline of events for study 1.

Instrumentation

\[ F_{\text{ENO}} \] was measured at an expiratory flow rate of 50 mL/s with a device designed to measure \( F_{\text{ENO}} \) (Niox Mino, Aerocrine, Solna, Sweden). Spirometry was measured in triplicate at each collection point with a spirometer (SDI SBG, Queset Medical, Brockton, Massachusetts). EBC was collected orally during tidal breathing for 7 min, at initial temperature of \(-20^\circ\text{C}\) with a sampling device (RTube, Respiratory Research, Austin, Texas), without nose clips. All samples underwent gas-standardization with research-grade oxygen for 10 min at 300 mL/min prior to pH measurement.

EBC pH was measured using an electrode (Orion 8220BNWP PerpHeCT Ross combination pH Micro Electrode, Thermo Scientific, Waltham, Massachusetts). The probe was calibrated in standard pH 4 and 7 buffers, and then verified in low-ionic-strength buffers of the same pH, and then pH of samples was measured immediately after gas-standardization.

Nebulizations were administered for 10 min using a nebulizer (Micromist, Hudson RCI/Teleflex Medical, Research Triangle Park, North Carolina), with a mouthpiece. The nebulizer was powered by 7 L/min of room air, a rate at which it averages an output mass median aerodynamic diameter of 2.1 \(\mu\text{m}\), according to manufacturer. The nebulizer cup was percussed periodically during medication delivery, to minimize residual volume. The residual volume was removed at the end of each 10-min inhalation. Oxygen saturation was obtained using a fingertip pulse oximeter (SET, Massimo, Irvine, California).

Study Medication Specifications

The alkaline glycine buffer was formulated by a compounding pharmacy under sterile conditions. Sodium chloride and sodium hydroxide were added to a solution of double deionized water and alkaline glycine, in order to attain appropriate isotonicity (0.9% weight/volume), osmolarity (338 mOsmoles), and pH (9.8) to prevent cough upon nebulization. After receipt of each batch of medication from the pharmacy, one vial was used to confirm pH and osmolarity.

Statistical Analyses

Outcome parameters post-treatment were compared with a pre-treatment baseline, using Wilcoxon rank-sum measures. Data are presented as median and interquartile range (IQR). A \( P \) value of \(<.05\) was considered significant. \( P \) values considered to be significant were not modified in the setting of multiple comparisons.

Results

Ten subjects (7 male, 3 female, mean age 33.2 y) performed the glycine inhalation in study 1. Eight subjects (6 male, 2 female, mean age 35.4 y) repeated the experiment 6 weeks later, inhaling standard normal saline instead of alkaline glycine. Two subjects had moved away during this time and were unable to complete this portion of the study. Ten subjects (4 male, 6 female, mean age 43.9 y) performed the second study. All of the subjects tolerated the inhaled glycine buffers. There were no ad-
verse events of any kind. The changes in measurements for the studied groups from baseline throughout the studies are reported in the Table. The median change in FENO from baseline after both glycine buffer and normal saline inhalation is reported in Figure 2. Vital signs were unchanged in all subjects throughout both studies. FENO levels decreased by a median of \(21.62\%\) (\(P = .004\)) after study 1 glycine inhalation, but there was no significant change in FENO in study 2. The largest decreases in FENO from baseline occurred in subjects with clinically elevated FENO levels in absolute terms at baseline (\(40\) ppb). Spirometry levels remained within normal clinical ranges for both studies (see Table). There was no adverse effect on our primary safety outcome variable, FEV\(_1\). There was a slight decrease of 5.11\% in FEF\(_{25-75}\%) after inhalation of study 1 glycine (IQR 12.579 to 0.292, \(P = .04\)), which was not seen in study 2, and also of 6.22\% in FVC after inhalation of study 2 glycine (IQR 2.16 to 6.17, \(P = .04\), see Table), which was not seen in study 1.

EBC pH increased by a median of 0.235 pH units after inhalation of the study 2 alkaline glycine buffer (IQR 0.56–0.03, \(P = .03\), Fig. 3), but had no statistically significant change after the study 1 treatments.

Table. Median Changes in Physiologic Measurements From Baseline

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>% Δ</th>
<th>(P^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>IQR</td>
<td>Median</td>
<td>IQR</td>
</tr>
<tr>
<td>Initial study - saline group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV(_1), L</td>
<td>4.08</td>
<td>4.53–3.74</td>
<td>4.24</td>
<td>4.56–3.68</td>
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<tr>
<td>FVC, L</td>
<td>5.06</td>
<td>5.58–4.88</td>
<td>5.12</td>
<td>5.51–4.9</td>
</tr>
<tr>
<td>FEV(_1)/FVC, %</td>
<td>80.06</td>
<td>82.18–76.38</td>
<td>81.69</td>
<td>81.90–78.62</td>
</tr>
<tr>
<td>F(_{\text{ENO}}), ppb</td>
<td>15</td>
<td>52–12</td>
<td>15.5</td>
<td>43.5–10.5</td>
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<tr>
<td>EBC pH</td>
<td>8.62</td>
<td>8.86–6.95</td>
<td>8.59</td>
<td>8.83–8.37</td>
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<tr>
<td>Initial study - glycine group</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>FEV(_1), L</td>
<td>3.97</td>
<td>4.47–3.46</td>
<td>3.99</td>
<td>4.25–3.52</td>
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<tr>
<td>FVC, L</td>
<td>4.96</td>
<td>5.51–4.61</td>
<td>5.04</td>
<td>5.45–4.54</td>
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<tr>
<td>FEV(_1)/FVC, %</td>
<td>78.55</td>
<td>83.19–72.82</td>
<td>79.44</td>
<td>81.68–72.08</td>
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<tr>
<td>FEF(_{25-75}%), %</td>
<td>3.72</td>
<td>4.89–2.83</td>
<td>3.53</td>
<td>4.58–2.84</td>
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<tr>
<td>F(_{\text{ENO}}), ppb</td>
<td>18.5</td>
<td>32–14</td>
<td>14.5</td>
<td>32–10</td>
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<tr>
<td>EBC pH</td>
<td>7.75</td>
<td>8.48–7.47</td>
<td>7.85</td>
<td>8.40–7.56</td>
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<tr>
<td>Second study - glycine group</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>FEV(_1), L</td>
<td>2.76</td>
<td>3.48–2.58</td>
<td>2.79</td>
<td>3.31–2.49</td>
</tr>
<tr>
<td>FEV(_1)/FVC, %</td>
<td>78.6</td>
<td>83.1–74.0</td>
<td>80.35</td>
<td>82.1–75.6</td>
</tr>
<tr>
<td>FEF(_{25-75}%), %</td>
<td>2.6</td>
<td>3.14–2.32</td>
<td>2.84</td>
<td>3.25–2.17</td>
</tr>
<tr>
<td>F(_{\text{ENO}}), ppb</td>
<td>23</td>
<td>43–16</td>
<td>21.5</td>
<td>38–18</td>
</tr>
<tr>
<td>EBC pH</td>
<td>7.71</td>
<td>8.27–7.17</td>
<td>8.34</td>
<td>8.62–7.36</td>
</tr>
</tbody>
</table>

* The \(P\) values have not been adjusted for multiple comparisons.
\(FEF_{25-75}\) = forced expiratory flow during the middle half of the FVC maneuver
\(F_{\text{ENO}}\) = fraction of exhaled nitric oxide
EBC = exhaled breath condensate

![Figure 2](image-url)
After inhalation of alkaline glycine, the subjects in study 2 did not experience a statistically significant change in FENO, but, in contrast, did demonstrate a marked increase in EBC pH. This finding supports the use of EBC pH to detect acidic ALF pH, as several of the subjects in this study demonstrated acidic EBC pH at baseline, consistent with known findings of low EBC pH in patients with disease (median 7.71, IQR 7.17–8.27). These subjects had known obstructive airway disease, and many were receiving inhaled corticosteroid therapy, which is known to decrease nitric oxide synthase activity and net nitrogen oxide production. Corticosteroid therapy decreases FENO at baseline, which likely explains the lack of an observed FENO effect in study 2.27

Our studies had several limitations. The primary focus was on the safety of the inhaled alkaline glycine buffer; therefore, several pieces of data that would have strengthened the secondary end points were not measured. In study 1 the healthy controls were not screened for atopy prior to enrollment. This could explain the differences noted in baseline FENO, EBC pH, and spirometry levels in the same subjects between the glycine treatment and the saline treatment. In study 1, EBC pH was collected only at baseline and after the final nebulization of alkaline glycine buffer. This was done to minimize delays between the 3 nebulizations. We used change in FENO after inhalation as a surrogate detector of airway pH changes at these time points, due to the rapidity of the testing (−11 s for FENO, compared to −7 min for EBC collection). Since study 1 was the first human trial of this alkaline glycine buffer, it was necessary to deliver the compound through serial small doses, in order to evaluate its safety and detect potential adverse reactions prior to delivery of a larger dose. Given the full tolerability of the inhaled buffer, we increased the concentration of buffer so that the next phase would receive only one treatment.

In study 2 we wished to evaluate the effects of inhaled alkaline glycine on patients with obstructive lung function: specifically, on the physiologic issue of air-flow limitation. Although heterogeneous, all enrolled subjects had documented airway obstruction on the study day. We did not determine their degree of airway hyper-responsiveness or responsiveness to β2 agonists at the time of the study. We did not test the patients for atopy, immunologic dis-
ease, unrecognized environmental/occupational illness, or for any of the other numerous factors that could contribute to their obstruction. This current study was simply examining safety in patients with air-flow obstruction. In future studies we will be evaluating the efficacy of inhaled alkaline glycine buffer in patients with obstructed lower airways, and will use classification schemes and semi-specific disease names such as “asthma” and “COPD,” to the extent that they are not misleading.

Also in study 2 we did not screen out subjects based on corticosteroid use. This may explain the lack of significant change in FENO results, since corticosteroid therapy decreases FENO at baseline. We also did not serially measure FENO and EBC pH for time points after inhalation of the alkaline glycine buffer. In previous studies the greatest reduction in FENO following inhalation of alkaline buffer occurred 15–60 min after inhalation. When using FENO to detect changes in airway pH, we recommend documenting and/or withholding corticosteroids prior to the study, and measuring FENO at several time points in the hour following the study intervention.

Although the inhaled medication evaluated in this study was a solution (not a colloid), the formulation was different from the physiologic saline used by the manufacturer to determine the 2.1 μm mass median aerodynamic diameter output of the nebulizer that was used in this study. It is possible that the mass median aerodynamic diameter output of the nebulizer of the study medication may be different from that of physiologic saline. This will be evaluated in future studies.

Conclusions

In conclusion, we report that the inhalation of isotonic alkaline glycine is safe in humans with or without obstructive airway disease, and that both EBC pH and FENO levels may be useful to indicate effective alkalization of the airways. Future studies should evaluate the effects of alkaline therapy on subjects with known acute airway pH disturbances. Perhaps the most interesting near-term use of inhaled alkaline glycine is to improve the absorption across the airway epithelium of certain therapeutic agents that carry a charge at acidic but not alkaline pH. Such therapeutics (including most β agonists and anticholinergics) achieve better passive and active transfer through the airway epitheliun—and therefore access to their smooth muscle target—when the ALF is alkaline. Because these medications are currently delivered at low pH, and they are mostly used during acute respiratory illnesses, when the airways are most likely to be acidic, the possibility of improving drug delivery by means of alkalization with glycine is currently undergoing clinical investigation.

REFERENCES

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