Breath sulfides and pulmonary function in cystic fibrosis


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We have determined the concentrations of carbonyl sulfide (OCS), dimethylsulfide, and carbon disulfide (CS2) in the breath of a group of cystic fibrosis (CF) patients and one of healthy controls. At the detection sensitivity in these experiments, room air always contained measurable quantities of these three gases. For each subject the inhaled room concentrations were subtracted from the time-coincident concentrations in exhaled breath air. The most significant differences between the CF and control cohorts in these breath-minus-room values were found for OCS. The control group demonstrated a net uptake of 250 ± 20 parts-per-trillion-by-volume (pptv), whereas the CF cohort had a net uptake of 110 ± 60 pptv (P = 0.00003). Three CF patients exhaled more OCS than they inhaled from the room. The OCS concentrations in the CF cohort were strongly correlated with pulmonary function. The dimethylsulfide concentrations in breath were greatly enhanced over ambient, but no significant difference was observed between the CF and healthy control groups. The net (breath minus room) CS2 concentrations for individuals ranged between +180 and −100 pptv. They were slightly greater in the CF cohort (+26 ± 36 pptv) vs. the control group (−17 ± 15 pptv; P = 0.04). Lung disease in CF is accompanied by the subsistence of chronic bacterial infections. Sulfides are known to be produced by bacteria in various systems and were therefore the special target for this investigation. Our results suggest that breath sulfide content deserves attention as a noninvasive marker of respiratory colonization.

bacterial emission | early detection | Pseudomonas aeruginosa | carbonic anhydrase | mucin sulfation

In the respiratory tract of cystic fibrosis (CF) patients, impairment of mucociliary clearance and innate defense mechanisms lead to susceptibility to chronic infections by opportunistic bacteria. These infections progress to chronic inflammation, bronchial obstruction, and, in ~80% of CF patients, eventual respiratory failure (1).†† Most CF patients are initially colonized by Staphylococcus aureus and/or Haemophilus influenzae (2)‡‡; however, by adulthood mucoid Pseudomonas aeruginosa emerges as the most prevalent CF pathogen (3). A positive response for P. aeruginosa is strongly associated with respiratory deterioration and mortality (4). A difficulty in treating Pseudomonas-positive patients is that over time the species transforms into a resistant mucoid variant (5). After the phenotypic transformation the infections become nearly impossible to eradicate (5). Early detection and antibiotic therapy has been promoted as a means to delay chronic P. aeruginosa colonization because several studies demonstrated delayed rates of reinfection when patients were treated early (6–9). Early detection of another less common but equally virulent species, Burkholderia cepacia, may also be beneficial because prompt isolation of positive patients might reduce the frequency of patient-to-patient transmission (10). Prophylactic antibiotic treatment to delay the acquisition of S. aureus, however, may actually enable earlier colonization by P. aeruginosa (11).

Current techniques to assess colonization include expectorated sputum, bronchoalveolar lavage, and oropharyngeal culture analyses. The first two methods are considered to be fairly accurate, but unfortunately they may not be able to detect Pseudomonas infections when they first occur (4). Moreover, obtaining sputum from children is difficult, and the bronchoalveolar lavage procedure is invasive and may not detect localized infections. For these reasons, new, noninvasive methods for the detection of CF respiratory bacteria, if proven effective, would be valuable clinical tools.

One possible, minimally invasive procedure is the analysis of trace gases in the breath of CF patients. Sulfides and other trace gases are known to be produced by bacteria (12, 13), including P. aeruginosa (14, 15) and B. cepacia (14), and the relative amounts of these gases seem to be characteristic of species and strain. For these reasons we hypothesized that sulfides might be elevated in the exhaled breath of CF patients. Here we report the results of a pilot study in which we measured concentrations, in the breath of a sample of CF patients and healthy controls, of three sulfides that are known to be produced by bacteria: carbonyl sulfide (OCS), dimethylsulfide (DMS), and carbon disulfide (CS2).

Materials and Methods

Subjects. Twenty CF patients (mean age, 17 years; range, 8–40 years; 8 females) and 23 healthy control subjects (mean age, 20 years; range, 9–37 years; 9 females) completed participation in the study. The difference in age between the two groups was not statistically significant. All CF patients had been previously diagnosed with CF according to criteria outlined by the CF Foundation. For each, their pulmonary disease was best classified as mild to moderate [forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) ≥ 50%; resting oxygen saturation ≥ 92%]. CF patients were excluded if there was evidence of acute pulmonary exacerbation at the time of their scheduled visit or if they had severe liver cirrhosis.

The healthy volunteers included in the study had no history of smoking, drug or alcohol abuse, or obesity and did not use any chronic medications (bronchodilators, antihypertensives, etc.). All subjects refrained from eating or drinking for at least 3 h before testing. Written informed consent was obtained from subjects or their legal guardians, and the protocol was approved by the University of California, Irvine Institutional Review Board.

Abbreviations: CF, cystic fibrosis; CI, confidence interval; CS2, carbon disulfide; DMS, dimethylsulfide; FEF25–75, forced expiratory flow between 25% and 75% of vital capacity; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; OCS, carbonyl sulfide; pptv, parts-per-trillion-by-volume.

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‡‡Cystic Fibrosis Foundation Patient Registry (2003) 2002 Annual Data Report to the Center Directors (Cystic Fibrosis Foundation, Bethesda).

§Cystic Fibrosis Foundation Patient Registry (2002) 2001 Annual Data Report to the Center Directors (Cystic Fibrosis Foundation, Bethesda).

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Exhaled Gas Collection. Exhaled gas for chemical analysis was collected by using an electropolished stainless steel apparatus consisting of a mouthpiece, valve, and 1.9-liter canister. The canisters were evacuated to \(<10^{-5}\) atmospheres. Subjects were instructed to inhale to total lung capacity, hold their breath for 5 s, and then exhale through the mouthpiece slowly to near residual volume in a 10-s exhalation. Each subject practiced the maneuver several times before the first breath collection, and all subjects were able to complete the maneuver without difficulty. The first 3 s of exhaled gas was vented to the room to assure collection of a predominantly alveolar sample. A total of four breath samples was collected from each subject. Some subjects were unable to fill canisters completely with one breath. For these subjects, two breaths were collected per sample. In those cases, the second breath was collected within 60 s of the first.

The samples were collected once every 15 min during a 1-h period. Room air samples also were collected at each of the four time points to account for ambient effects. The concentrations were then averaged for the four time points. A total of 172 breath and 172 room air samples (43 subjects \(\times 4\) time points per subject \(\times 1\) breath plus 1 ambient at each time point) were collected and analyzed.

Samples obtained from CF patients and healthy controls contained similar percentages of CO\(_2\) (CF 5.1 \(\pm\) 0.2\% vs. healthy 4.8 \(\pm\) 0.2\%; \(P = 0.07\)). This result indicates that the alveolar fraction of samples acquired from the two cohorts was similar. The mean intrasubject standard deviation in CO\(_2\) concentration was 0.29\% in CF patients and 0.34\% in healthy controls, indicating that the maneuvers were reproducible.

Sample pressures also were measured before analysis to verify that the canisters had been completely filled and that subambient storage pressures were not important. Breath sample pressures were very close to those of the ambient environment, averaging 740 torr.

Correction for Ambient. The concentrations of gases in human breath are influenced by those in the ambient environment (room air). To correct for these effects, we subtracted the background concentrations from time-equivalent breath concentrations. Each of these three sulfide gases is found in outside air and was present outside the sampling location in approximately the same range of trace concentrations.

Breath Sample Storage and Analysis. Breath samples were stored in their collection canisters for an average of 14 days before chemical analysis. In experiments that were conducted during the time interval of this study we found that the concentrations of the sulfide gases reported in this work are not affected by length of storage. Sulfide depletion caused by partitioning into condensed water in breath samples was not an issue because of the small Henry’s constants of these gases and because the volume of water vapor that condenses is not substantial (\(\sim 0.05\) ml).

The samples were quantified on an ultra-trace gas analytical system developed in our laboratory. At the detection sensitivity of this analytical system, several hundred individual chemical components have been observed, \(\sim 200\) of them have been identified, and \(\sim 100\) have been quantitatively assayed. These components include hydrocarbons, organic molecules containing oxygen, chlorine, bromine, fluorine, and/or nitrogen, all of them being atmospheric components routinely encountered in outside air when the minimum sensitivity of detection is \(\sim 1\) parts-per-trillion-by-volume (pptv) or smaller.

From each sample a 268-ml (standard temperature and pressure) aliquot was passed through a stainless steel loop containing glass beads and maintained at \(\sim 196^\circ\)C. This procedure preconcentrated the relatively less volatile components, including OCS, DMS, and CS\(_2\), while allowing the bulk of the air to pass through the loop to the vacuum pump. The trapped compounds then were revolatilized by immersing the sample loop in hot water (\(\sim 80^\circ\)C), and subsequently flushed into a helium carrier flow. The sample flow then was quantitatively divided into six separate streams. The compounds in each stream were chromatographically separated on a particular individual column and directed to a single specific detector. OCS, DMS, and CS\(_2\) were quantified with a quadrupole mass selective detector that detected the sulfur fragments specific to each molecule. DMS also was quantified in two other streams with different chromatographic columns equipped with flame ionization detectors. The results were quantitatively consistent with those obtained from the quadrupole mass selective detector.

Each breath and room gas concentration was determined by comparing the sample chromatogram gas peak area with the gas peak area in the working standard chromatogram. In our regular laboratory practice, a working standard was analyzed after every eight sample runs. The concentrations of OCS, DMS, and CS\(_2\) in the working standard were derived by using a standard that was calibrated at the National Center for Atmospheric Research (Boulder, CO). For a complete description of the sample analysis techniques used in this study, see Colman et al. (16).
in group means. The F-score method was used to determine homogeneity of variance. Simple linear regression was used to determine Pearson’s correlation coefficients, slopes, and intercepts for relationships between pairs of measurement level variables. The significance of each correlation coefficient was evaluated by Student’s t test. Confidence intervals (CIs) for correlation coefficients were determined by using the Fisher’s z-score transformation technique. All variables were assumed to be normally distributed. Joint normality was assumed in the linear regression analyses. All statistical tests involving gas concentrations were performed under room-corrected gas concentrations. P > 0.05 was considered statistically insignificant.

Results

OCS. The measured concentrations of OCS in exhaled breath and ambient samples were consistent with one another across the four time points. The mean intrasubject coefficient of variation was 8% in breath and 4% in ambient air. The distributions of OCS concentrations for CF patients and healthy subjects are displayed in Fig. 1. Exhaled breath-minus-room OCS concentrations were negative for all healthy subjects and for the majority of CF patients; that is, the concentrations of the gas were generally higher in the inhaled room air than in the exhaled breath air. In 3 of the 20 CF patients, however, exhaled OCS exceeded the inhaled quantity. OCS was less negative in the CF patients (−110 ± 60 pptv; mean ± 0.95 CI) than in healthy controls (−250 ± 20 pptv; P = 0.00003). OCS values also varied to a greater extent between subjects in the CF group (CF intersubject = 120 pptv vs. healthy intersubject = 46 pptv; P = 0.00003, where intersubject is an estimate of the population intersubject standard deviation). OCS was significantly less negative in the 15 CF patients who were taking DNase than the 5 CF patients who were not (−75 ± 67 vs. −210 ± 71 pptv; P = 0.005). We believe that this result originates from the fact that the patients that were not taking DNase were essentially asymptomatic. Their average FEV1 score was 104%, indicating that their respiratory bacterial burden was small. Breath OCS did not significantly correlate with gender, weight, height, or drugs other than DNase. Average OCS room air concentrations fell between 550 and 720 pptv. It should be emphasized that OCS has been present at similar concentrations in the atmosphere for centuries, and therefore exposure to the gas is routine both for CF patients and for the control group (and for all of the rest of us as well).

OCS and Pulmonary Function. Fig. 2A–D illustrates the relationship between breath OCS and one of the four indices of pulmonary function: FEV1%, FVC%, (FEV1/FVC)%, and FEF25–75%, respectively. In these illustrations, the index is plotted against OCS for the CF patients (Left) and the healthy controls (Right); each data point represents one subject’s data. Breath OCS concentrations were significantly and inversely correlated with all four indices in the CF population and were not correlated with any of the indices in the healthy subject population. In effect, subjects with poorer pulmonary function tended to have greater breath OCS concentrations. The regression and correlation coefficients describing these relationships are presented in Table 3. The tightest correlation observed was between FEV1% and OCS (R² = 0.56; P = 0.0002). FEF25–75% exhibited the largest percent decrease per pptv increase of OCS, decreasing by 21% (R² = 0.95; CI: 10–31%) for every 100 pptv increase in breath OCS.

Dimethylsulfide. DMS concentrations were more than a factor of 50 greater in breath samples than room air samples, indicating a substantial bacterial or physiological source active in both CF patients and controls. DMS concentrations in the CF group were not statistically different from those in the control group (4,780 ± 1,350 vs. 3,920 ± 680 pptv; P = 0.25). Concentrations of DMS in breath samples were more consistent across the four time points than in ambient air because the latter were much closer to the limit of detection (breath intersubject = 9%, room intersubject = 20%). The distributions of DMS concentrations for CF patients and healthy subjects are displayed in Fig. 3. Like OCS, DMS concentrations also varied to a greater extent between subjects in the CF group (CF intersubject = 2,890 pptv vs. healthy intersubject = 1,570 pptv; P = 0.007). DMS concentrations did not significantly correlate with gender, weight, height, pulmonary function, or drugs. DMS room air concentrations fell between 40 and 375 pptv, a range that is commonly observed for this gas. (DMS is much more chemically reactive than OCS in the atmosphere, resulting in a greater range in ambient concentrations.)
**CS₂**. CS₂ concentrations exhibited moderate consistency in breath and room samples (breath $\sigma_{\text{intrasubject}} = 21\%$, room $\sigma_{\text{intrasubject}} = 14\%$). The distributions of CS₂ concentrations for CF patients and healthy subjects are displayed in Fig. 4. Individual subject CS₂ concentrations, after subtraction of ambient room concentrations, were below zero in the majority of the control subjects and in half of the CF patients. Overall, CS₂ concentrations were slightly greater in the CF population than in the controls ($26 \pm 38$ vs. $-17 \pm 15$ pptv; $P = 0.04$). Like DMS and OCS, CS₂ concentrations varied to a greater extent between subjects in the CF group (CF $\sigma_{\text{intersubject}}$...
Breath Sulfides and Respiratory Colonization. In Table 4 we present the average breath sulfide concentrations for CF patients who were classified as positive for \textit{S. aureus} and/or \textit{P. aeruginosa} at the time of the study and those that were classified as negative for these organisms. We are unable to report any statistically significant differences in breath sulfides between these CF patient subpopulations. It is important to state that respiratory cultures were not collected at the time of breath sampling. Classifications into these subgroups were based on the patients’ most recent respiratory culture results as indicated in their medical records. Colonization status is known to change over time, and respiratory culture results can be inaccurate; therefore, we cannot be certain that all of the CF patients were classified correctly.

**Discussion**

In this initial study we measured the breath concentrations of three sulfide gases known to be produced by bacteria, OCS, DMS, and CS$_2$, in a group of CF patients and healthy control subjects. We compared the measured sulfide concentrations of the two groups and assessed the relationship between these concentrations and pulmonary function. We have suggested that the breath concentrations of OCS might be elevated in CF patients because of the increased presence of bacteria in their lungs.

The results of these measurements indicate that breath OCS concentrations are significantly enhanced in most CF patients and that these concentrations are inversely correlated with several indices of pulmonary function. All of the healthy subjects and 17 of 20 CF subjects exhibited negative breath concentrations of OCS (after subtracting the concentrations in room air) indicating net uptake of the gas during respiration. The increased variability for

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**Table 3. Regression/correlation coefficients describing the observed linear relationships between four standard pulmonary function indices and OCS in CF patients and controls**

<table>
<thead>
<tr>
<th>PFT index</th>
<th>Slope, mean ± 95% CI per pptv</th>
<th>Intercept, mean ± 95% CI</th>
<th>Correlation ($R^2$), mean (95% CI)</th>
<th>Significance of correlation ($P$ value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1%</td>
<td>CF $-0.14 \pm 0.06$</td>
<td>$61 \pm 7$</td>
<td>$0.56 (0.18-0.81)$</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>Control $0.1 \pm 0.1$</td>
<td>$120 \pm 6$</td>
<td>$0.11 (0.02-0.46)$</td>
<td>0.13</td>
</tr>
<tr>
<td>FVC%</td>
<td>CF $-0.10 \pm 0.05$</td>
<td>$75 \pm 6$</td>
<td>$0.45 (0.09-0.75)$</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Control $0.1 \pm 0.1$</td>
<td>$114 \pm 6$</td>
<td>$0.05 (0.00-0.37)$</td>
<td>0.32</td>
</tr>
<tr>
<td>FEF$_{25-75}$%</td>
<td>CF $-0.2 \pm 0.1$</td>
<td>$43 \pm 12$</td>
<td>$0.50 (0.13-0.78)$</td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td>Control $0.1 \pm 0.2$</td>
<td>$125 \pm 8$</td>
<td>$0.08 (0.00-0.46)$</td>
<td>0.13</td>
</tr>
<tr>
<td>(FEV1/FVC)%</td>
<td>CF $-0.07 \pm 0.03$</td>
<td>$80 \pm 4$</td>
<td>$0.52 (0.15-0.79)$</td>
<td>0.0003</td>
</tr>
<tr>
<td></td>
<td>Control $-0.01 \pm 0.07$</td>
<td>$89 \pm 3$</td>
<td>$0.005 (0.00-0.15)$</td>
<td>0.76</td>
</tr>
</tbody>
</table>

PFT, pulmonary function test.

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Fig. 3. Plotted are the distributions of breath DMS concentrations in CF and healthy subjects. Individual subject means (averaged across the four time points) are represented by circles. Group means are represented by dashes. The y-bars attached to the group means indicate the 0.95 CIs for the population means.

Fig. 4. Plotted are the distributions of breath CS$_2$ concentrations in CF and healthy subjects. Individual subject means (averaged across the four time points) are represented by circles. Group means are represented by dashes. The y-bars attached to the group means indicate the 0.95 CIs for the population means.

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= 81 pptv vs. healthy $\sigma_{\text{intersubject}} = 35$ pptv; $P = 0.0003$). CS$_2$ was significantly greater in CF patients who were using the drug DNase (DNase 40 ± 50 pptv vs. no DNase $-17 \pm 36$ pptv; $P = 0.04$). As in the case of OCS, we believe that this difference simply parallels the severity of the CF symptoms because the patients that were not using DNase were those who had normal pulmonary function. Breath CS$_2$ did not significantly correlate with gender, weight, height, or drugs other than DNase. CS$_2$ room air concentrations fell between 3 and 29 pptv.
this gas in the CF population parallels the wide range of pulmonary disease severities in the sample.

Exhaled CS₂ was greater in the CF population, but there was not a significant correlation between CS₂ and lung function. OCS, DMS, and CS₂ varied to a greater extent between CF patients than between healthy individuals. DMS was elevated in the CF patients compared with controls, but not significantly so.

Although we have not directly established that the enhanced OCS in CF patients is of bacterial origin, several studies have demonstrated an inverse relationship between pulmonary function and respiratory bacterial load. For example, Ordonez et al. (17) reported increased FEV₁ and decreased P. aeruginosa and S. aureus in sputum in 40 CF patients after administration of i.v. antibiotics. Regelmann et al. (18) and Ramsey et al. (19) observed similar reductions in P. aeruginosa and increased pulmonary function after antibiotic therapy. Ramsey et al. (19) also observed reductions in total bacterial load in sputum.

Another reason to suspect that the OCS disparity might be related to bacteria is that the high-molecular-weight oligosaccharide chains in CF respiratory mucin contain a considerably increased abundance of sulfate esters (20–22). There is evidence that this enhanced mucin sulfation supplies resistance to utilization by P. aeruginosa and B. cepacia (23). However, Jansen et al. (24) demonstrated that strains of P. aeruginosa and B. cepacia, isolated from CF patients, can desulfate mucin through mucin sulfatase. After desulfation, mucin may become susceptible to bacterial proteinases and glycosidases generating amino acids and carbohydrates for bacterial consumption (24, 25). To our knowledge, however, OCS has not been reported as a product of mucin degradation.

The net uptake of OCS observed in both subject groups may be a result of the metabolism of OCS by the zinc metalloenzyme carbonic anhydrase. Chengelis and Neal (26) demonstrated that rat hepatocytes and bovine erythrocyte carbonic anhydrase both readily convert OCS to carbon dioxide, hydrogen sulfide, and probably thiolsulfate. Acetzolamide, a carbonic anhydrase inhibitor, was shown to inhibit the process in both experiments. Investigators have questioned whether the distribution and/or activity of carbonic anhydrase, which is found throughout the human body, may be altered in CF patients (27, 28). Recently, Fanjul et al. (29) showed that the targeting of the carbonic anhydrase isoform CA IV to plasma membranes in human pancreatic duct cells, which expressed the ΔF508 CFTR mutation, is disrupted. Thus, an intriguing explanation of the OCS data might be that a functional impairment in carbonic anhydrase may limit OCS uptake and metabolism in CF patients’ lungs, contributing, along with bacterial production of the gas, to generally higher levels within the alveolar and/or airway gas. Further investigation is needed to determine the exact origin of the OCS disparity between the CF and healthy populations.

In summary, we measured breath OCS, DMS, and CS₂ in a group of CF patients and healthy controls. OCS and CS₂ were significantly enhanced in the breath of CF patients, and OCS concentrations were inversely correlated with lung function in the CF group. Although the exact origins of these disparities are uncertain, possibilities include increased bacterial load, disparities in physiological uptake, and differences in the chemical makeup of respiratory mucin in CF patients. We conclude that breath sulfide content, especially as OCS, deserves attention as a potential noninvasive marker of respiratory bacterial colonization in CF. Furthermore, we suggest that the ultra-trace gas breath analysis techniques that were used in this study possess wide-ranging clinical potential.

We thank Yunsoo Choi for technical contributions to the sample analyses. We thank the General Clinical Research Center at the University of California, Irvine. This work was supported in part by National Institutes of Health Grants HD 20639 and HL 080947, the Cystic Fibrosis Foundation, and the Joan Irvine Smith and Athalie R. Clarke Foundation. M.B. was supported by a research grant from the University of Aachen, Medical Faculty, Program for Young Scientists.

Table 4. Comparisons of average breath sulfide concentrations in the CF patient subgroups defined by respiratory colonization status

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>n</th>
<th>OCS, mean ± 0.95 CI (pptv)</th>
<th>CS₂, mean ± 0.95 CI (pptv)</th>
<th>DMS, mean ± 0.95 CI (pptv)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>14</td>
<td>6</td>
<td>−90 ± 70</td>
<td>−100 ± 100</td>
</tr>
<tr>
<td>S. aureus</td>
<td>9</td>
<td>11</td>
<td>−100 ± 100</td>
<td>−80 ± 60</td>
</tr>
</tbody>
</table>

References: