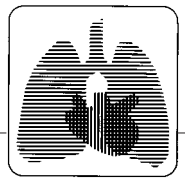
A Pilot Study of the Effect of Inhaled Buffered Reduced Glutathione on the Clinical Status of Patients With Cystic Fibrosis

Clark Bishop, Valerie M. Hudson, Sterling C. Hilton and Cathleen Wilde

Chest 2005;127;308-317
DOI 10.1378/chest.127.1.308



preliminary report

A Pilot Study of the Effect of Inhaled Buffered Reduced Glutathione on the Clinical Status of Patients With Cystic Fibrosis*

Clark Bishop, MD, FCCP; Valerie M. Hudson, PhD; Sterling C. Hilton, PhD; Cathleen Wilde, BS

Study objectives: **To assess the impact of inhaled, buffered reduced glutathione (GSH) on clinical indicators of cystic fibrosis (CF) pathophysiology.**
Design and patients: **A randomized, double-blind, placebo-controlled pilot study was conducted over an 8-week period. Nineteen subjects, age 6 to 19 years, with CF status documented by positive sweat chloride test results (> 60 mEq/L) were recruited for the trial. After matching on age and sex, 10 patients were randomly assigned to the treatment group and 9 patients to the placebo group. Primary outcomes were FEV_1, FVC, forced expiratory flow at 25 to 75% of vital capacity, and peak flow; secondary outcomes were body mass index, 6-min walk distance, and self-reported cough frequency, mucus production/viscosity/color, wellness, improvement, and stamina.**
Interventions and analysis: **Treatment was buffered GSH, and placebo was sodium chloride with a hint of quinine. The total daily dose of buffered GSH was approximately 66 mg/kg of body weight, and the total daily dose of placebo was approximately 15 mg/kg of body weight (quinine, 25 to 30 μg/kg). Doses were distributed across four inhalation sessions per day and spaced 3- to 4-h apart. General linear mixed models were used to analyze the data. The final sample size was nine subjects in the treatment group and seven subjects in the placebo group.**
Results: **Mean change for peak flow was − 6.5 L/min for the placebo group and + 33.7 L/min for the GSH group ($p = 0.04$), and self-reported average improvement on a scale from 1 to 5 (1 being much worse and 5 being much better) was 2.8 for placebo and 4.7 for GSH ($p = 0.004$). Of the 13 primary and secondary outcomes examined, 11 outcomes favored the treatment group over the placebo group ($p = 0.002$), indicating a general tendency of improvement in the GSH group. No adverse events in the treatment group were noted.**
Conclusion: **This pilot study indicates the promise of nebulized buffered GSH to ameliorate CF disease, and longer, larger, and improved studies of inhaled GSH are warranted.**
(CHEST 2005; 127:308–317)

Key words: aerosolized; bronchiectasis; cystic fibrosis; glutathione; lung function; mucolysis; oxidative stress

Abbreviations: BMI = body mass index; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; ELF = epithelial lining fluid; $FEF_{25–75}$ = forced expiratory flow at 25 to 75% of vital capacity; GSH = reduced glutathione; GSH+GSSG = total glutathione; GSSG = glutathione disulfide; IL = interleukin; mOsm = milliosmol

Cystic Fibrosis (CF) is a genetic disease affecting some 30,000 individuals in North America, and > 200,000 worldwide. CF is caused by the autosomal recessive mutation of the CF transmembrane conductance regulator (CFTR) protein, which is located on chromosome 7. In nonaffected individuals, the CFTR protein is transcribed and then migrates to and creates a channel in the cell membrane; this channel allows for the egress from the cell of certain anions. In patients with CF, this channel is missing or defective.[1,2]

CF pathology is characterized by excessive inflam-

mation in the respiratory and GI systems. Focusing on the respiratory system, patients with CF have thick mucus secretions, and colonization with bacteria occurs at an early age. The usual course of CF in

For editorial comment see page 12

the respiratory system is chronic infection that leads to airways obstruction, bronchiectasis, and eventual respiratory failure, with approximately 95% of patients with CF eventually dying from respiratory failure. Although much CF research has been on the altered chloride efflux from the CFTR channel,[1,2] other anions, such as bicarbonate (HCO_3^-) and reduced glutathione (GSH) [L-gamma-glutamyl-L-cysteinyl-glycine] also pass through the CFTR to exit the cell.[3,4]

While CF pathology is well documented, the means by which the CF mutation causes disease is not well understood. It has been hypothesized that the altered distribution of GSH in patients with CF provides a significant link between CF genetics and CF pathology.[5] The mechanism of this potential link can be understood by first understanding the function of GSH in normal individuals.

GSH in Epithelial Lining Fluid

GSH performs several important functions in the epithelial lining fluid (ELF), particularly of the lung. First, GSH functions as the primary water-soluble antioxidant in the ELF, by directly or enzymatically scavenging hydrogen peroxide, hyperchlorous acid, and other free radicals.[6] In this process, GSH is oxidized to glutathione disulfide (GSSG). Body compartments normally exposed to high oxidative stress have correspondingly high levels of GSH on the epithelial surface, and a high redox ratio (GSH/GSSG).[7] For example, the ELF of the lungs contains 140 times the serum concentration of GSH, and the redox ratio is $>$ 9:1.[8] Second, GSH helps to maintain proper mucus viscosity and facilitates cell signaling through the cleavage of disulfide bonds.[5,9,10] Third, after oxidation to GSSG, GSH reversibly bind to certain proteins, protecting them from irreversible damage in times of oxidative and nitrosative stress.[11]

In patients with CF, research[3,12–14] has shown that lung epithelial cells have normal total glutathione (GSH+GSSG) but exhibit significantly diminished efflux of GSH through CFTR channels at the apical surface. This diminished efflux results in profoundly decreased GSH+GSSG as well as decreased redox ratio in the ELF of the CF lung; total glutathione can be 10 to 50% of normal, and the redox ratio can be as low as 3:1[15,16]; therefore, patients with CF receive neither the full antioxidant nor mucolytic benefits of GSH.

GSH in the Immune System

GSH also plays multiple, pivotal roles in the normal immune system. First, inflammation is closely tied to both the GSH/GSSG ratio and GSH+GSSG.[5,17] A decrease in either amount, even in the absence of infection, leads to the transcription of nuclear factor-κB, with a cascade of proinflammatory cytokines, such as tumor necrosis factor-α, interleukin (IL)-8, IL-6, IL-1a, and others following.[17–20] Second, normal intracellular levels of GSH are essential for important immune system cell functions such as chemotaxis, phagocytosis, appropriate apoptosis, oxidant burst, microtubule integrity, cell signaling, antigen presentation, release of lysosomal enzymes, and other bactericidal activities.[9,21–23] Third, the GSH system profoundly affects the nitric oxide system, with GSH deficiency inducing a reduction in available nitric oxide for use in bactericidal, bronchodilation, and cell-signaling functions.[24–26]

In CF, reduced levels of GSH+GSSG and a diminished GSH redox ratio could help explain the chronic and excessive inflammation in the respiratory system. Although epithelial cells have a defective or missing CFTR channel, immune cells, such as neutrophils, possess redundant anion channels, including those of the multidrug resistance-associated protein family, which allows for the egress of GSH from these cells[27]; however, research[28] has shown that neutrophils in CF also have diminished levels of GSH. It is hypothesized that part of the altered functionality of the neutrophils could be explained by the constant demand placed on them by the diminished GSH levels in the ELF.[5]

Therefore, chronic GSH deficiency in the CF lung could explain the paradox of an overactive immune system (chronic inflammation) that is nevertheless ineffective in eradicating bacteria (chronic infection). This paradox together with the loss of antioxidant protection and thickened mucus lead to a CF lung that is seriously compromised by genetically induced GSH transport dysfunction (Fig 1).

*From Utah Valley Regional Medical Center (Dr. Bishop), and Brigham Young University (Drs. Hudson and Hilton, and Ms. Wilde), Brigham Young University, Provo, UT.
All funding provided by the Utah Valley Institute of Cystic Fibrosis.
Manuscript received March 1, 2004; revision accepted May 14, 2004.

Correspondence to: Clark Bishop, MD, FCCP, 1034 North 500 West, Provo, UT 84605-0390; e-mail: UVCBISHO@ihc.com

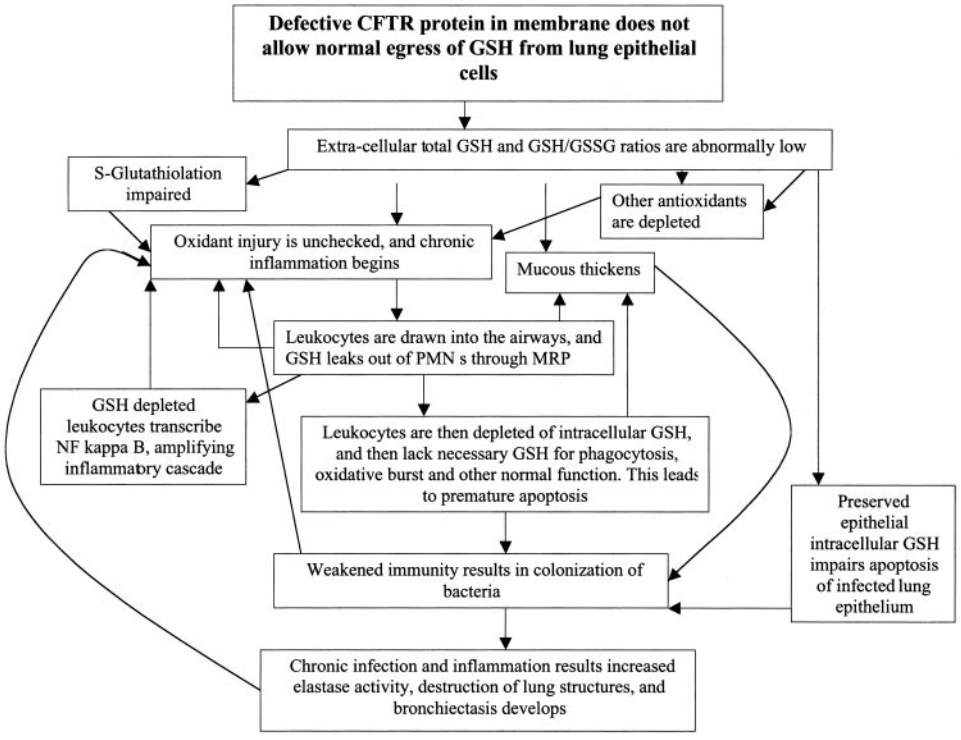


FIGURE 1. Role of ELF GSH depletion in the pathophysiology of CF. PMN = polymorphonuclear leukocytes; NF = nuclear factor; MRP = multidrug resistance-associated protein.

While it has been known since 1987[29] that GSH is depleted in respiratory ELF in CF, some[30] have argued that the low levels of GSH are secondary to chronic inflammation. Research[3,12–14] allows us to say that the decreased GSH in the ELF of the CF lung, while aggravated by inflammation and infection characteristic of CF, is nevertheless caused in the first place by CFTR mutation. This new understanding constitutes an important link between CF genetics and CF pathophysiology, and it raises a question about the therapeutic potential of augmenting GSH by exogenous means.

Efforts to augment ELF GSH levels in the CF lung have been undertaken. In addition to one *in vitro* trial using CF human bronchial epithelial cells lines,[31] there have been nine human trials on inhaled GSH, and one trial using sheep.[32–40] No serious adverse effects were reported in any of the nine human *in vivo* trials. Two of the nine human *in vivo* trials involved patients CF. The first trial[31] demonstrated GSH in the lower airway following nebulization, and the study demonstrated decreased hypersensitivity of CF leukocytes to inflammatory stimuli. However, this trial was not placebo controlled, and it was only of 3 days in duration, with a maximum dose of 600 mg bid, and few clinical variables were examined. The second trial[40] also demonstrated GSH in the lower airways following nebulization, and found increased FEV_1 and altered lymphocyte profile. But again, this trial was not placebo controlled, and the longest duration of use was 2 weeks, also with a fairly small dose (450 mg tid).

Given the theoretical promise of GSH therapy in patients with CF and the previous findings of inhaled GSH,[31–40] we conducted a placebo-controlled pilot study of longer duration with a higher daily dose of a buffered GSH solution. The objective was to assess the impact of GSH on clinical indicators of CF pathophysiology. Specific hypotheses included the following: (1) primary indicators involving lung function, FEV_1, FVC, forced expiratory flow at 25 to 75% of vital capacity ($FEF_{25–75}$), and peak flow, would be positively affected by this intervention; (2) secondary outcomes representing other markers of CF disease would be positively affected, including body mass index (BMI), 6-min walk distance, and self-reported cough frequency, mucus production/viscosity/color, wellness, improvement, and stamina; and (3) no adverse effects would be attributable to this intervention (no pattern of worsening of bacterial cultures/counts, or other types of adverse events). All outcomes and their scales are defined in Table 1, and qualitative scales of subjective measures were all categorized as secondary outcomes. The study rationale, design, methods, and protocol were reviewed

Table 1—*Baseline Demographic and Clinical Characteristics of the Two Treatment Groups

Variables	Placebo (n = 9)	GSH (n = 10)
Clinical characteristics		
FVC	87.2 (23.60)	89.3 (17.18)
FEV_1	79.7 (24.72)	81.6 (18.17)
$FEF_{25–75}$	67.7 (33.82)	68.4 (24.49)
Peak flow	285.5 (118.46)	379.5 (105.46)
BMI	17.9 (2.34)	18.5 (2.13)
6-min walk distance, feet	2,823.0 (414.33)	3,101.1 (578.30)
Usual stamina	2.8 (0.63)	3.1 (0.60)
General wellness	2.7 (0.48)	3.1 (0.60)
Cough frequency	2.7 (0.67)	2.3 (0.87)
Sputum amount	1.8 (0.79)	1.7 (1.22)
Sputum viscosity	2.0 (0.47)	1.7 (1.12)
Sputum color	2.7 (0.75)	2.5 (1.54)
Demographics		
Age, yr	12.9 (4.9)	13.3 (4.1)
Weight, lb	88.5 (31.4)	92.3 (29.1)
Male gender, %	60	67

*Data are presented as mean (SD) unless otherwise indicated.

and approved by the Utah Valley Regional Medical Center Institutional Review Board, Provo, UT.

Materials and Methods

Subjects

Nineteen subjects aged 6 to 19 years with CF status documented by positive sweat chloride test results (> 60 mEq/L) were recruited for the trial. Patients were recruited between March 2002 and June 2002, through personal contact, newspaper advertisement, and Internet groups. Participants were not paid, but were reimbursed for travel expenses > 60 miles round-trip. Patients were excluded for the following reasons: (1) positive culture finding for *Burkholderia cepacia* at any time, (2) history of hemoptysis or pneumothorax, (3) FEV_1 < 30% of predicted, or (4) severe asthma. Informed consent was obtained after explaining the study and its purpose to the participants and parents. Patients of legal age provided informed consent, and parents of minor children provided informed consent. Minor children added their own assent, after an age-appropriate explanation of the trial was given. Sample size for the trial was based on a power analysis of FEV_1 with an anticipated effect size of 15% improvement.

Experimental Protocol

Randomization: Patients were first paired by age and sex, and then each member of the pair was randomly assigned to the treatment or placebo groups. Just before the trial began, one patient in the placebo group dropped out. No member of the clinical team was involved in the coding or assignment to treatment/placebo groups, and both patients and the clinical team remained blinded to treatment group assignment throughout the trial. Nonclinical researchers involved in any coding were only provided patient identification numbers, not patient names.

Trial Structure: The trial duration was 8 weeks, with enrollment occurring between late June and early July 2002 depending on patient availability for the first examination. Each patient was seen by the clinical team at the Utah Valley Regional Medical Center at the beginning and end of the trial. Primary and secondary outcomes (Table 1) were collected at these visits with the exception of peak flow; patients were asked to perform peak flows at least twice daily throughout the trial using a peak flowmeter (Personal Best; Respironics; Murrysville, PA). Baseline bacterial culture samples and counts were either collected at the first visit or extracted from medical records for the 3 months prior to the first visit; bacterial cultures and counts were also collected at the end of the trial.

Patients were instructed to continue taking all currently prescribed medication and treatments, and to phone or e-mail immediately if there were any adverse events. Study protocol outlined that all adverse events were to be promptly reported to the principal investigator who would assess the clinical situation and recommend any necessary deviation from the treatment regimen. Also, clinical researchers phoned or e-mailed patients biweekly to monitor side effects and to encourage compliance, and they asked patients midway through the trial of their perceptions of any change in clinical status. Finally, patients were asked to record daily in a logbook changes in status, timing of treatments, and peak flows. A subjective assessment of compliance was made after the trial ended based on logbook entries.

During clinic visits, patients were segregated according to bacterial status. Patients with mucoid *Pseudomonas aeruginosa* or methicillin-resistant *Staphylococcus aureus* were segregated from each other and other patients, *ie*, seen in separate rooms with all equipment and rooms cleaned between patients. In addition, patients with methicillin-resistant *S aureus* were gowned, and their testing was done at the end of the day.

Materials and Dosage

GSH The treatment group received capsules containing reduced GSH buffered with sodium bicarbonate. Each capsule contained 300 mg of GSH and 72 mg of 100% pure sodium bicarbonate. The capsules were formulated by Theranaturals, Inc. (Orem, UT). The GSH was manufactured using good manufacturing practice by the Kohjin Company (Tokyo, Japan). Nelson Laboratories (Salt Lake City, UT), an independent testing laboratory, performed limulus amebocyte lysate tests for endotoxins on the lot of GSH used in the study. No endotoxins were found by tests with sensitivity thresholds < 0.015 endotoxin units per milligram. This lot of GSH was also tested and found negative for *S aureus*, *P aeruginosa*, Salmonella species, *Escherichia coli*, and fungi. The purity of this lot of GSH was assayed using high-pressure liquid chromotography, with purity measuring 98.8% GSH with heavy metals not > 5 ppm, iron not > 5 ppm, and arsenic not > 1 ppm. The pH of the buffered GSH in solution tested at 5.2, and average osmolality of one capsule dissolved in 3.75 mL of sterile water was measured at 469 milliosmol (mOsm)/kg, producing a slightly hypertonic solution of 1.4% (physiologic osmolality being 280 to 310 mOsm/kg).

Placebo: The placebo group received capsules containing sodium chloride with a hint of quinine. Since GSH has a distinct taste and odor, quinine was added to the placebo in order to create a distinct taste and odor. Each capsule contained approximately 68 mg (range, 68 to 102 mg) or 1.16 mmol of sodium chloride, and 125 μg or 0.4 μmol/L of quinine. The capsules were formulated by Theranaturals, Inc. The pH of the placebo in solution tested at 4.9, and average osmolality of one capsule dissolved in 3.75 mL of sterile water was measured at 1,360 mOsm/kg, producing approximately a 4% hypertonic saline solution.

Dosage: Total daily dose of buffered GSH for each patient was approximately 66 mg/kg of body weight, and total daily dose of

placebo for each patient was approximately 15 mg/kg of body weight (25 to 30 μg/kg of quinine). These doses were distributed across four inhalation sessions per day, and patients were asked to space these sessions 3- to 4-h apart. For the first week of treatment, patients were instructed to use one fourth of the recommended total dosage, and in the second week to use one half of the recommended total dosage. After the second week, patients were instructed to use the full daily total dosage. The primary purpose of this incremental dosage regimen was to monitor for adverse events at lower dosage levels.

Each patient was individually instructed regarding the number of capsules to use for each session and to dilute each capsule using 3.75 mL of sterile water. All patients received a portable compressor/nebulizer (Omron NE-C21 Comp-Air Elite; Omron Healthcare; Vernon Hills, IL), whose particle size tested at 6 μm. For a given session during the day, patients were instructed to make the solution, place it in the nebulizer, and inhale it using a face mask until it was gone. Patients were instructed to always make a fresh solution for each session. Laboratory experiments show that freshly made buffered GSH solution is stable for up to 5 h with GSH in solution, being 97 ± 0.1% in the reduced form.[29]

Data Analysis

Differences between posttrial and baseline outcomes were analyzed using a general linear mixed model that allowed for correlation between outcomes within the age/sex pair used for randomization. For peak flow, *baseline* was defined as the average of all measurements taken the first 5 days of the trial, and *post* was defined as the average of all measurement taken the last 5 days on treatment. All analyses were conducted on a modified intent-to-treat basis, *ie*, regardless of compliance to the treatment regimen, all patient outcomes were included in the analysis except when patients were hospitalized or were missing data. Patients who were hospitalized were excluded from analysis because their posttrial lung function outcomes would be clearly influenced by their hospitalization treatment. Also, when patients were missing data for a particular outcome, they were excluded from the analysis of that outcome; however, missing data were minimal. Two patients in the GSH group did not record daily peak flow data; and at the posttrial clinic visit, one patient in the placebo group did not provide data on sputum viscosity and color, and two patients in the GSH group did not provide data on sputum color.

Participant Flow

The recruitment process and patient experience is summarized in Figure 2. Fifty-eight patients inquired about the trial, 23 patients were excluded on the basis of the exclusion criteria noted above, and 16 patients chose to not participate. Nineteen patients were enrolled in the trial: 10 in the treatment group and 9 in the placebo group. After the trial commenced, three patients (two receiving placebo and one receiving GSH) were hospitalized due to nonacute pulmonary exacerbations that were unrelated to participation in the study. Since hospitalized patients were excluded from data analyses, study results are based on data from seven patients receiving placebo and nine patients receiving GSH.

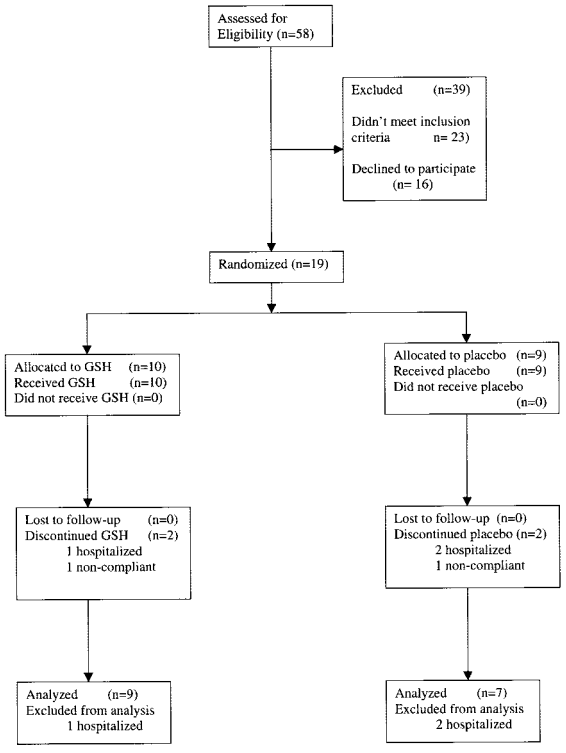


FIGURE 2. Patient flow.

RESULTS

Baseline patient demographic and clinical characteristics are given in Table 2. As expected after randomization, no statistically significant differences were found between the placebo and baseline groups for any of the baseline demographic and clinical characteristics. The variance component for the pairs was estimated to be zero in most analyses, which suggests that there was very little variability between the pairs, and that age/sex matching was probably unnecessary in this trial. This, of course, may not be the case in trials conducted for longer periods of time and with more patients.

Adverse events recorded during the trial are provided in Table 3. Three patients were hospitalized (two patients receiving placebo, and one patient receiving GSH) due to nonimprovement of conditions present at baseline. During the trial, one participant complained to the principal investigator of chest pain, and another complained of cough and nasal irritation. Both were instructed to reduce their dosage to one half the number of capsules and diluent. Both patients were in the placebo group, and these were the only patients to verbally report any adverse event during the trial. The other adverse events reported in Table 3 were extracted from comments written in the daily logbooks. In general, the symptoms listed in Table 3 are commonly found in individuals with CF, and no apparent differences in these symptoms were found between the two treatment groups. Also, an analysis of the number

Table 2—*Primary and Secondary Outcomes Measured at Baseline and End of Trial, and Scales on Which They Are Measured*

Outcomes	Scales
Primary	
FEV_1	% predicted of FEV_1
FVC	% predicted of FVC
$FEF_{25–75}$	% predicted of $FEF_{25–75}$ of FVC
Peak flow*	Liters per minute using flow meter
Secondary	
BMI	
6-min walk test	Distance (feet) walked in 6 min
Sputum color	Self-report on following scale: 1 = clear, 2 = white, 3 = yellow, 4 = green, 5 = brown, 6 = blood streaked
Sputum amount	Self-report on scale: 1 = scant, 2 = < 1 teaspoon, 3 = > 1 teaspoon
Sputum viscosity	Self-report on scale: 1 = very thin, 2 = slightly sticky, 3 = very sticky
Cough frequency	Self-report on scale: 1 = no cough, 2 = infrequent, 3 = several times a day, 4 = every hour
General wellness	Self-report on scale: 1 = poor, 2 = fair, 3 = good, 4 = excellent
Usual stamina	Self-report on scale: 1 = poor, 2 = fair, 3 = good, 4 = excellent
Improvement†	Self-report on scale: 1 = significantly worse, 2 = a bit worse, 3 = about the same, 4 = a bit better, 5 = significantly better

*Measured by patient at least twice daily throughout trial period. Baseline measure is average of first 5 days of trial; end of trial measure is average of last 5 days of trial.
†Only measured at end of trial.

Table 3—*Type of Adverse Events During Trial for Each Treatment Group**

Events	Placebo (n = 9)	GSH (n = 10)
Hospitalization for nonacute pulmonary exacerbations	2	1
Rhinitis/sinusitis	3	2
Cough	3	4
Pharyngitis	4	4
Stomach pain/cramps	4	1
Headache	2	4
Chest tightness/bronchospasm	3	1
Nose bleed	3	2
Shortness of breath	2	1

*Data are presented as No. of patients.

and severity of species identified in bacterial cultures indicated no significant differences between the treatment and placebo groups over the course of the trial.

The mean differences (postbaseline) between the GSH and placebo groups for both primary and secondary outcomes are presented in Table 4. Also found in Table 4 are 95% confidence intervals for the effect size and p values corresponding to hypothesis tests of no effect size. Changes in sample size due to missing data are noted. The effect size for all four primary outcomes favored the GSH group; however, none were statistically different except for peak flow ($p = 0.04$). For peak flow, the placebo group essentially had a small average decline of − 6.5 L/min, and the GSH group improved on average by 33.7 L/min. For the $FEF_{25–75}$ measure, there was a slight decline (− 5.0%) in the placebo group, and a slight improvement (1.2%) in the GSH group. For the FVC and FEV_1 measures, both groups declined on average over the 8-week period; however, the declines were slightly less for the GSH group compared to the placebo group.

For the nine secondary outcomes, the self-reported overall improvement score was statistically different ($p = 0.004$) between the treatment groups. The placebo group reported feeling on average "about the same," while the GSH group reported feeling on average "much better." Two of the secondary outcomes, general wellness ($p = 0.09$) and cough frequency ($p = 0.20$), tended toward statistical significance. The GSH group improved on average 0.4 on a 4-point scale more than the placebo in terms of self-reported general wellness, and the self-reported frequency of cough declined slightly in the GSH group but remained the same in the placebo group. The remaining six outcomes were not significantly different between the two groups, although four of the six outcomes favored the GSH group.

One GSH patient was grossly noncompliant, *ie*, stopped the treatment after the first 5 days of the trial. In order to obtain a clearer picture of the potential effect of GSH, the data were analyzed excluding this patient. These results are not reported since they are very similar to those in Table 4 except for two noteworthy differences: (1) the difference in average cough frequency declined by 0.6 (scale 1 to

Table 4—*Modified Intent-to-Treat Analysis: Estimated Average Differences Between End of Trial and Baseline Measures for Two Treatment Groups, and Estimated Effect Size Associated With Test of No Effect Size*

Outcomes	Placebo (n = 7)		GSH (n = 9)		Effect Size (GSH − placebo)	95% Confidence Interval	p Value
	0	(SD)	0	(SD)			
FVC	− 3.3	(4.3)	− 2.7	(9.7)	0.6†	− 7.8–9.1	0.88
FEV_1	− 3.7	(3.8)	− 2.8	(10.4)	0.9†	− 7.4–9.3	0.81
$FEF_{25–75}$	− 5.0	(11.7)	1.2	(14.8)	6.2†	− 8.0–20.9	0.38
Peak flow	− 6.5	(32.0)	33.7	(35.2) [n = 7]	40.2†	1.0–79.4	0.04
BMI	0.0	(0.6)	0.1	(1.1)	0.1†	− 0.9–1.0	0.90
6-min walk distance	30.9	(158.1)	57.8	(123.7)	26.9†	− 124.0–178.0	0.71
Usual stamina	0.6	(0.5)	0.4	(1.0)	− 0.2	− 1.0–0.8	0.77
General wellness	0.4	(0.5)	0.8	(0.7)	0.4†	− 0.1–1.0	0.09
Cough frequency	0.0	(0.6)	− 0.4*	(0.7)	− 0.4*†	− 1.1–0.3	0.20
Sputum amount	0.0	(0.6)	0.1*	(0.7)	0.1*	− 0.5–0.7	0.71
Sputum viscosity	0.2	(1.2) [n = 6]	− 0.2*	(0.9)	− 0.4*†	− 1.4–0.6	0.42
Sputum color	0.7	(2.2) [n = 6]	− 0.1*	(1.1) [n = 7]	− 0.8*†	− 2.8–1.2	0.39
Improvement	2.8	(1.2)	4.7	(0.6)	1.9†	0.8–3.0	0.004

*A negative value indicates an improved condition.

†Favors the GSH group: p = 0.002 that 11 of 13 patients would favor GSH if there were no positive GSH effect.

4) in the GSH group, and was statistically different (p = 0.03) from the placebo group; and (2) the average difference in $FEF_{25–75}$ between the GSH and placebo groups increased to 8.4%, which tended toward statistical significance (p = 0.24).

Discussion

This pilot study yielded encouraging results that warrant a closer examination by means of larger, longer clinical trials. Small airway function improved in the GSH group, as seen in the significant improvement in peak flows and the tendency toward significance of $FEF_{25–75}$ in the ancillary compliance analysis. Because two subjects in the GSH group did not record peak flow data, the peak flow comparison is comparable to the compliance analysis. While the effect size in peak flow is relatively small (40.2 L/m), improvement in small airway function is noteworthy because research[41] in CF pathophysiology suggests that changes in peripheral air flow precede changes in FEV_1 and FVC in this disease. In addition to small airway function, two self-reported secondary indicators significantly improved in the GSH treatment group: subjective sense of improvement (p = 0.004), and subjective assessment of cough frequency in the ancillary compliance analysis (p = 0.03). A measure of subjective general wellness tended toward significant improvement as well (p = 0.09). Finally, none of the outcomes significantly favored the control group over the GSH group.

This pilot study demonstrates significant differences in a few important end points, and the results are consistent with the hypothesis that GSH does improve clinical indicators in CF patients. Why then were not more indicators, especially FEV_1 and FVC, significantly different between the two groups? There are two opposing possibilities that are worth considering. First, inhaled GSH may not be an effective treatment for CF symptoms. If this is the case, then the significant differences found in this study represent statistical type I errors, and further study of inhaled GSH is unnecessary. However, this explanation is improbable given the small p values and the multiple significant differences that were observed in this study. The second possible explanation for finding few significant differences in this study is that inhaled GSH is an effective treatment for CF symptoms, but limitations in this pilot study prevented the actual effect of GSH to occur and to be detected. In order to fully consider this second explanation, it is important to discuss the limitations of the study.

An important limitation of this study is its short duration. The study lasted only 8 weeks, and patients were only at full dosage for the last 6 weeks of the trial. It is possible that more time receiving treatment is required before detectable improvements in lung function occur. Another limitation is the small sample size. Measures of lung function exhibit natural variability; therefore, it is possible that if group differences exist, they will only be detectable with larger samples. Also, the patients in this study were fairly healthy patients (average FEV_1 was 80.7%, and average FVC was 88.3%), which limits the amount of improvement that can be observed. Another limitation is the placebo solution. The smallest amount of the placebo mixture that could be reliably encapsulated at the facilities available to us was 100 mg. This resulted in a 4% hypertonic saline solution, and the

use of hypertonic saline of 3 to 12% has been demonstrated to have therapeutic value to patients with CF.[42–44] Therefore, it is possible that the observed effects in these data are smaller than they would be if a true placebo solution had been used.

Another important limitation of this study is that the optimal dose of inhaled GSH is still unknown. Most of the previous *in vivo* studies[31–40] of inhaled GSH used 600 mg/d; however, neither explanation nor justification for this dose has been given. We used the following rationale to arrive at the dosage levels in this study. Given that research[8,15,28] has shown reduced levels of GSH in blood serum, lymphocytes, and WBCs, and others[13,16] have shown a 50% reduction in export of GSH, and given that Meister[45] has shown that a normal 150-lb male subject synthesizes 10 g of GSH per day, our dosage schedule was based on a 50% replacement of daily, synthesized GSH in the body. While previous *in vivo* trials[31,40] have demonstrated adequate delivery and a half-life of several hours of inhaled GSH, not all of the prepared solution reaches the lung due to leakage and other factors.[40] Also, given the recent findings[46] that normal mice respond to oxidative stimuli with a threefold increase in ELF GSH, and that CFTR knockout mice lack this response, it is possible that even higher doses are required to achieve effective treatment. Clearly, further dosing studies are necessary in order to identify the optimal dose.

If inhaled GSH is effective and the study limitations discussed above muted or prevented the actual effect of GSH to occur and/or to be detected, then only a few outcomes might show a significant GSH effect while a general tendency or syndrome of improvement might exist collectively across the outcomes. A simple way to conceptualize a general tendency or syndrome of improvement is to assume that there is no GSH effect. Under this assumption, one would expect the estimated effect size to favor the control and GSH groups with equal probability. Between the primary and secondary outcomes, there are 13 clinical end points, of which 11 favored the GSH treatment group. Under the assumption of no GSH effect, the probability of observing 11 of 13 effects favoring the GSH group is 0.002. This probability is extremely small, which suggests that in this 8-week period we are seeing a syndrome of improvement in the GSH treatment group affecting a broad spectrum of symptoms, including airway function, sputum characteristics, weight gain, cough frequency, and stamina measured in distance walked, among others (Table 4, column 3).

This general syndrome of improvement is consistent with the significant difference ($p = 0.004$) between the groups in their subjective assessment of improvement: the GSH group reported feeling much better, while the control group reported feeling about the same. It is also consistent with information garnered from exit interviews with study participants. Several patients in the GSH group reported an amelioration of other symptoms that we did not investigate, including improvement in sinus inflammation and nasal mucus, improved stool characteristics, and decreased need for pancreatic enzymes. Given that some significant differences were found despite the study limitations and given that the data show a general tendency toward improvement, it seems that inhaled GSH has the potential to be an effective treatment for many CF symptoms. Certainly there is sufficient promise that longer and larger studies of inhaled GSH are warranted.

In general, the GSH treatment was well tolerated by the patients, and none of the patients in the GSH group notified the principal investigator of serious adverse events. Based on verbal complaints and logbook entries, the GSH and placebo groups appeared to have similar frequency and severity of reported adverse events. Also, there were no significant changes or differences in the number and severity of species identified in bacterial cultures between the treatment and placebo groups (the noncompliant GSH patient cultured rare *Burkholderia gladioli* at the end of the 8-week trial; the pathogenicity of *B gladioli* in patients with CF is unknown); subsequent culture findings from this patient have been negative for *B gladioli*.[47] Of course, additional trials will be required to verify the safety of inhaled GSH, especially in fragile patients who were excluded from the pilot study (culturing *B cepacia*, history of hemoptysis/pneumothorax, $FEV_1 < 30\%$ predicted, or severe asthma component.)

It should be noted that secondary GSH deficiency has been observed and perhaps plays an aggravating role in several other respiratory diseases, including COPD,[48] ARDS,[49] idiopathic pulmonary fibrosis of nonsmokers,[50] AIDS-related respiratory disease,[51] idiopathic interstitial pneumonia,[52] idiopathic respiratory distress syndrome,[53] and diffuse fibrosing alveolitis.[54] Therefore, this therapeutic approach might be useful in diseases other than CF.

In conclusion, while there is emerging evidence that primary GSH deficiency is an important element of the CF pathology, it must be understood that CF disease is certainly multifactorial in origin. Short of a genetic cure, however, effective treatment of CF should address the GSH deficiency. The findings from this pilot study are consistent with the hypothesis that augmentation of ELF GSH does significantly improve clinical indicators in patients with CF. Specifically, we observed a spectrum of fairly ameliorative results, most noteworthy of which

was significant improvement in small airway function. We believe it is clear that further investigation is required to determine the extent of the effectiveness of GSH on CF symptoms; therefore, higher powered studies of longer duration should be conducted that examine a larger array of clinical indicators, including inflammatory mediators and exhaled breath characteristics.

ACKNOWLEDGMENT: We thank Travis Dastrup, MS, Columbia University School of Medicine; Melissa Redding, Brigham Young University; Colleen Saxey, RN, Brigham Young University; and Nicholas Bishop, Brigham Young University, for assisting in conducting the trial, and for data input and analysis.

References

1 Gibson RL, Burns JL, Ramsey BW. Pathophysiology and management of pulmonary infections in cystic fibrosis. Am J Respir Crit Care Med 2003; 168:918–951
2 Skach WR, ed. Cystic fibrosis: methods and protocols. Totowa, NJ: Humana Press, 2002
3 Linsdell P, Hanrahan JW. Glutathione permeability of CFTR. Am J Physiol 1998; 44:C323–C326
4 Ko SB, Shcheynikov N, Choi JY, et al. A molecular mechanism for aberrant CFTR-dependent HCO_3^- transport in cystic fibrosis. EMBO J 2002; 21:5662–5672
5 Hudson VM. Rethinking cystic fibrosis pathology: the critical role of abnormal reduced glutathione (GSH) transport caused by CFTR mutation. Free Radic Biol Med 2001; 30:1440–1461
6 Kelly FJ. Glutathione: in defence of the lung. Food Chem Toxicol 1999; 37:963–966
7 Smith CV, Jones DP, Guenther TM, et al. Compartmentation of glutathione: implications for the study of toxicity and disease. Toxicol Appl Pharmacol 1996; 140:1–12
8 Cantin AM, North SL, Hubbard RC, et al. Normal alveolar epithelial lining fluid contains high levels of glutathione. J Appl Physiol 1987; 63:152–157
9 Peterson JD, Herzenberg LA, Vaquez K, et al. Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns. Proc Natl Acad Sci U S A 1998; 95: 3071–3076
10 Robinson MK, Rodrick ML, Jacobs DO, et al. Glutathione depletion in rats impairs T-cell and macrophage immune function. Arch Surg 1993; 128:29–34
11 Klatt P, Lamas S. Regulation of protein function by S-glutathiolation in response to oxidative and nitrosative stress. Eur J Biochem 2000; 267:4928–4944
12 Kogan I, Ramjeesingh M, Kidd J, et al. CFTR directly mediates nucleotide-regulated glutathione flux. EMBO J 2003; 22:1981–1989
13 Velsor LW, van Heeckeren A, Day BJ. Antioxidant imbalance in the lungs of cystic fibrosis transmembrane conductance regulator protein mutant mice. Am J Physiol Lung Cell Mol Physiol 2001; 281:L31–L38
14 Gao L, Kim KJ, Yankaskas JR, et al. Abnormal glutathione transport in cystic fibrosis airway epithelia. Am J Physiol Lung Cell Mol Physiol 1999; 277:L113–L118
15 Roum JH, Buhl R, McElvaney NG, et al. Systemic deficiency of glutathione in cystic fibrosis. J Appl Physiol 1993; 75: 2419–2424
16 Finder JD, Engman CL, Kagan VE, et al. Broncoalveolar lavage fluid from patients with cystic fibrosis have diminished levels of reduced glutathione [abstract]. Pediatr Pulmonol 2003; 25(suppl):158
17 Rahman I, MacNee W. Regulation of redox glutathione levels and gene transcription in lung inflammation: therapeutic approaches. Free Radic Biol Med 2000; 28:1405–1420
18 Sen CK, Khanna S, Reznick AZ, et al. Glutathione regulation of tumor necrosis factor-α-induced NF-κB activation in skeletal muscle-derived L6 cells. Biochem Biophys Res Commun 1997; 237:645–649
19 Haddad JJ, Oliver RE, Land SC. Antioxidant/pro-oxidant equilibrium regulates HIF-1α and NF-κB redox sensitivity: evidence for inhibition by glutathione oxidation in alveolar epithelial cells. J Biol Chem 2000; 275:21130–21139
20 Tanaka C, Kamata H, Takeshita H, et al. Redox regulation of lipopolysaccharide(LPS)-induced interleukin-8 (IL-8) gene expression mediated by NF-κB and AP-1 in human astrocytoma U373 cells. Biochem Biophys Res Commun 1997; 232:568–573
21 Boxer LA, Oliver JM, Speilberg SP, et al. l. Protection of granulocytes by vitamin E in glutathione synthetase deficiency. N Engl J Med 1979; 301:901–905
22 Galietta LJ, Folli C, Marchetti C, et al. Modification of transepithelial ion transport in human cultured bronchial epithelial cells by interferon-γ. Am J Physiol Lung Cell Mol Physiol 2000; 278:L1186–L1194
23 Kelly TJ, Drumm ML, Inducible nitric oxide synthase expression is reduced in cystic fibrosis murine and human airway epithelial cell lines. J Clin Invest 1998; 102:1200–1207
24 Chen G, Wang SH, Warner TD. Regulation of iNOS mRNA levels in endothelial cells by glutathione, a double-edged sword. Free Radic Res 2000; 32:223–234
25 Vos TA, Goor H, Tuyt L, et al. Expression of inducible nitric oxide synthase in endotoxemic rat hepatocytes is dependent on cellular glutathione status. Hepatology 1999; 29:421–426
26 Singh SP, Wishnok JS, Keshive M, et al. The chemistry of the S-nitrosoglutathione/glutathione system. Proc Natl Acad Sci U S A 1996; 93:14428–14433
27 Wright SR, Boag AH, Valdimarsson G, et al. Immunohistochemcial detection of multidrug resistance protein in human lung cancer and normal lung. Clin Cancer Res 1998; 4: 2279–2289
28 Tirouvanizam RN, Tijoe I, Moss RB, et al. Multiparameter study of CF blood and lung leukocytes using 3-laser, 11-color cytometry. Pediatr Pulmonol 2001; 22:271–272
29 Crystal RG. Aerosol preparation of glutathione and a method for augmenting glutathione level in lungs. US Patent 5 238 683. August 24, 1993.
30 Hull J, Vervaart P, Grimwood K, et al. Pulmonary oxidative stress response in young children with cystic fibrosis. Thorax 1997; 52:557–560
31 Roum JH, Borok Z, McElvaney NG, et al. Glutathione aerosol suppresses lung epithelial surface inflammatory cell-derived oxidants in cystic fibrosis. J Appl Physiol 1999; 87:438–443
32 Buhl R, Vogelmeier C, Critenden M, et al. Augmentation of the glutathione in the fluid lining the epithelium of the lower respiratory tract by directly administering glutathione. Proc Natl Acad Sci U S A 1990; 87:4063–4067
33 Buhl R, Holroyd K, Borok Z, et al. Reversal of the glutathione deficiency in the lower respiratory tract of HIV-seropositive individuals by glutathione aerosol therapy [abstract]. Clin Res 1990; 38:596A
34 Holroyd KJ, Buhl R, Borok Z, et al. Correction of glutathione deficiency in the lower respiratory tract of HIV-seropositive individuals by glutathione aerosol treatment. Thorax 1993; 48:985–989
35 Borok Z, Buhl R, Grimes GJ, et al. Effect of glutathione aerosol on oxidant-antioxidant imbalance in idiopathic pulmonary fibrosis. Lancet 1991; 338:215–216

36 Marrades RM, Roca J, Barbera JA, et al. Nebulized glutathione induces bronchoconstriction in patients with mild asthma. Am J Respir Crit Care Med 1997; 156:425–430
37 Bernorio S, Pecis M, Zucchi A, et al. Glutathione in bronchial hyperresponsiveness. J Aerosol Med 1996; 9:207–213
38 Testa B, Mesolella M, Testa D, et al. Glutathione in the upper respiratory tract. Ann Otol Rhinol Laryngol 1995; 104:117–119
39 Bagnato GF, Gulli S, DePasquale R, et al. Effect of inhaled glutathione on airway response to "Fog" challenge in asthmatics. Respiration 1999; 66:518–521
40 Griese M, Ramakers J, Krasselt A, et al. Improvement of alveolar glutathione, lung function, but not oxidative state in cystic fibrosis. Am J Respir Crit Care Med 2004; 169:822–828
41 Tiddens HA. Detecting early structural lung damage in cystic fibrosis. Pediatr Pulmonol 2002; 34:228–231
42 Daviskas E, Robinson M, Anderson SD, et al. Osmotic stimuli increase clearance of mucus in patients with mucociliary dysfunction. J Aerosol Med 2002; 15:331–341
43 Wark PA, McDonald V. Nebulised hypertonic saline for cystic fibrosis. Cochrane Database Syst Rev 2000; (2):CD001506
44 Robinson M, Hemming AL, Regnis JA, et al. Effect of increasing doses of hypertonic saline on mucociliary clearance in patients with cystic fibrosis. Thorax 1997; 52:900–903
45 Meister A. Glutathione metabolism. Methods Enzymol 1995; 251:3–7
46 Day BJ, van Heeckeren AM, Min E, et al. Role for cystic fibrosis transmembrane conductance regulator protein in glutathione response to bronchopulmonary Pseudomonas infection. Infect Immun 2004; 72:2045–2051
47 LiPuma J. Burkholderia and emerging pathogens in cystic fibrosis. Respir Crit Care Med 2003; 24:681–692
48 Rahman I, McNee W. Lung glutathione and oxidative stress: implications in cigarette-induced airway disease. Am J Physiol 1999; 277:L1067–L1088
49 Moss M, Guidot DM, Wong-Lambertina M, et al. The effects of chronic alcohol abuse on pulmonary glutathione homeostasis. Am J Respir Crit Care Med 2000; 161:414–419
50 Rahman I, Shwarska E, Henry M, et al. Systemic and pulmonary oxidative stress in idiopathic pulmonary fibrosis. Free Radic Biol Med 1999; 27:60–68
51 Pacht ER, Diaz P, Clanton T, et al. Alveolar glutathione fluid decreases in asymptomatic HIV-seropositive individuals over time. Chest 1997; 112:785–787
52 Kitamura S. The 74th Annual Meeting President Lecture: pathogenesis and therapy of acute lung injury [in Japanese]. Kekkaku 1999; 74:693–697
53 Boda D, Nemeth I, Pinter S. Surface tension, glutathione content, and redox ratio of the tracheal aspirate fluid of premature infants with IRDS. Biol Neonate 1998; 74: 281–288
54 Behr J, Degenkolb B, Maier L, et al. Increased oxidation of extracellular glutathione by bronchoalveolar inflammatory cells in diffuse fibrosing alveolitis. Eur Respir J 1995; 8: 1286–1292

A Pilot Study of the Effect of Inhaled Buffered Reduced Glutathione on the Clinical Status of Patients With Cystic Fibrosis

Clark Bishop, Valerie M. Hudson, Sterling C. Hilton and Cathleen Wilde

Chest 2005;127;308-317
DOI 10.1378/chest.127.1.308