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Case Study

Improvement in clinical markers in CF patients using a reduced glutathione regimen: An uncontrolled, observational study

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Abstract

CFTR mutation, which causes cystic fibrosis (CF), has also recently been identified as causing glutathione system dysfunction and systemic deficiency of reduced glutathione (GSH). Such dysfunction and deficiency regarding GSH may contribute to the pathophysiology of CF. We followed 13 patients (age range 1–27 years) with cystic fibrosis who were using a regimen of reduced glutathione (GSH), including oral glutathione and inhaled buffered glutathione in an uncontrolled, observational study. Dosage ranged from 66–148 mg/kg/day in divided doses, and the term examined was the initial 5.5 months of GSH use (45 days of incrementally adjusted dose, plus 4 months of use at full dosage). Baseline and post-measurements of FEV1 percent predicted, BMI percentile, and weight percentile were noted, in addition to bacterial status and pulmonary exacerbations. Significant improvement in the following clinical parameters was observed: average improvement in FEV1 percent predicted ($N=10$) was 5.8 percentage points ($p<0.0001$), average weight percentile ($N=13$) increased 8.6 points ($p<0.001$), BMI percentile ($N=11$) improved on average 1.22 points ($p<0.001$). All patients improved in FEV1 and BMI, if measured in their case; 12 of 13 patients improved in weight percentile. Positive sputum cultures of bacteria in 11 patients declined from 13 to 5 ($p<0.03$) with sputum cultures of *Pseudomonas aeruginosa* becoming negative in 4 of 5 patients previously culturing PA, including two of three patients chronically infected with PA as determined by antibody status. Use of a daily GSH regimen appears to be associated in CF patients with significant improvement in lung function and weight, and a significant decline in bacteria cultured in this uncontrolled study. These findings bear further clinical investigation in larger, randomized, controlled studies.

Keywords: Inflammation; FEV1; BMI; Weight percentile; *Pseudomonas aeruginosa*

1. Introduction

There has been increasing interest in the role of glutathione in the pathophysiology of cystic fibrosis. Since the discovery that the CFTR channel was the major mechanism of GSH efflux into the extracellular milieu of the lung from lung epithelial cells and that this efflux was severely compromised in cystic fibrosis (CF) [1–6], resulting in glutathione system dysfunction and systemic deficiency of reduced glutathione (GSH) in CF, researchers have begun to explore what role glutathione system dysfunction plays in cystic fibrosis disease. Not only have in vitro studies proven useful in the development of new hypotheses in this regard [7–17], but several clinical trials of GSH or GSH precursors such as NAC have resulted in improvement in clinically-relevant markers in CF patients [18–23].

2. Methods and cases

We followed 13 CF patients (age range 1–27 years; 7 male; 11 Italian patients (cases 1–11) and 2 American patients (cases 12–13)) with cystic fibrosis who were using a regimen of reduced glutathione (GSH), including oral glutathione and inhaled buffered glutathione, in an uncontrolled observational study. No patient had used GSH prior to this study. These patient histories were selected due to the recording of clinical markers at the temporal intervals needed for comparison, as

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Table 1
Dosing regimen for Italian (#1–11) and American (#12–13) patients

	Italian patients	American patients
Nebulizer	Pari e-Flow	Pari LC_Star or LC-Plus
Dosage	40 mg/kg/day by mouth, and 1800 mg/day by inhalation	66 mg/kg/day divided into 3–4 doses, with at least one dose inhaled
Dosage protocol	45 days of incrementally increasing dose to full dose, beginning with 30 mg bid to 900 mg bid for inhalation.	45 days of incrementally increasing dose to full dose, beginning with one-quarter of the full dose
Oral GSH source	GSH Caps and GSH-Curc Caps (Theranaturals)	GSH Caps and GSH-Curc Caps (Theranaturals)
Inhaled GSH source	TAD (Italy) diluted in 4 L of $NAHCO_3^-$, 1 mEq/L to obtain pH of 7.5, with osmolarity of 290 mM/L OR [a] Reduced L-Glutathione Plus Sodium Bicarbonate (Theranaturals) diluted in 3.75 L sterile water to obtain pH of 5.2 with osmolarity of 469 mM/L	Reduced L-Glutathione Plus Sodium Bicarbonate (Theranaturals) diluted in 3.75 L sterile water to obtain pH of 5.2 with osmolarity of 469 mM/L

[a] Only one formulation of inhaled GSH was used with each Italian patient; patient preference determined source.

well as reliability of information regarding dosage and date of initiation of the GSH regimen.

Patients did not modify any of their prescribed CF therapeutic regimen, but merely added GSH to that regimen. Patients requested to take GSH, having individually assessed this treatment. The two physicians involved asked to observe clinical markers of these patients, which request was fulfilled in most cases. All cases used a combination of oral and inhaled glutathione, as outlined in Table 1. Only pharmaceutical grade GSH was used by the patients, and inhaled glutathione was buffered to be of relatively neutral pH.

All thirteen patients had clinical markers assessed three months before baseline, at baseline, and 5.5 months after baseline. Three patients had one or more missing indicators. All patients except case 4 were pancreatic insufficient. Patient characteristics at 3 months before baseline, baseline, and 5.5 months after baseline are provided in Table 2A; additional patient characteristics at baseline and 5.5 months post-baseline are provided in Table 2B. For patients #1–11, data on these clinical markers were available three months prior to baseline; for patients #12–13, weight three months prior to baseline was available. These clinical markers were quite stable during this three month period. For example, the change in FEV1 predicted ranged from −2% to 2% with an average of −0.1%; the change in weight (kg) ranged from −0.5 to 0.5 kg with an average of −0.01 kg; and 8 patients had no change in bacteria cultured in their sputum while 3 patients had one additional bacteria at baseline. Therefore, the changes observed over the 5.5 month study period do not appear to be part of any trend toward improvement that started prior to GSH use.

3. Side effects

In three cases, mild side effects were noted. In Case 2, a slight fever appeared on days 33–35, which spontaneously disappeared without intervention. In Case 7, chest tightness was felt on days 15–16, but disappeared without intervention. In Case 11, diarrhea appeared on days 3–5, which resolved without intervention. ALT measurements at baseline and 5.5 months after baseline were collected for patients #1–11 to assess change in liver function (Table 2B). The average change in ALT was −3.72 IU/L (range 9 to −20), suggesting no hepatotoxic effects from this regimen.

4. Results

Clinical markers for the individual patients measured 5.5 months after the GSH regimen began are shown in Table 2A and B. With the exception of certain missing indicators for

Table 2A
Patient characteristics and clinical markers at 3 months before baseline, baseline, and 5.5 months after baseline

Case #, gender, age (years)	Mutations	FEV1 (% predicted)			Weight (kg)			Weight (sex–age percentile)			Sputum cultures (type of bacteria) [a]		
		T_{-3}	T_0	$T_{5.5}$	T_{-3}	T_0	$T_{5.5}$	T_{-3}	T_0	$T_{5.5}$	T_{-3}	T_0	$T_{5.5}$
1, M, 8	DF508/DF508	58	56	64	22	22	24	17	13	31	SA,PA [b]	SA,PA [b]	SA
2, F, 11	DF508/?	65	64	71	28.5	28	30	9	5	6	PA	PA	none
3, F, 19	DF508/G1244E	47	48	55	43	43	48	3	3	9	PA [b]	PA [b]	PA
4, M, 5	DF508/R347P	NA	NA	NA	17	17.5	19	33	33	40	SA	SA	none
5, M, 24	W1282G/G542X	60	58	70	51	51.5	57	3	3	7	BC	BC	BC
6, F, 14	3659delC/?	70	71	72	42	42	49	20	17	41	none	SA	none
7, F, 8	DF508/DF508	66	66	69	21	20.5	23	12	6	14	SA	SA,H	none
8, F, 7	Y1182X/G1244E	73	72	75	21	20.5	21.5	35	23	21	none	none	none
9, M, 27	DF508/2183delAA	38	38	44	53	53	58.5	3	3	10	PA [b]	PA [b]	SA
10, M, 22	2183delAA/?	24	25	29	57	57	60.5	7	7	15	AX	AX	AX
11, M, 15	DF508/R347H	42	44	51	50.5	50	54	30	24	32	PA	SA,PA	none
12, M, 1	NA	NA	NA	NA	10	10	12	9	5	25	NA	NA	NA
13, F, 1	DF508/DF508	NA	NA	NA	7.5	8	9.5	0	0	3	NA	NA	NA

NA — data not available.
T_{-3} — 3 months prior to baseline; T_0 — baseline; $T_{5.5}$ — 5.5 months after baseline.
[a] Bacteria codes: SA = *Staphylococcus aureus*, PA = *Pseudomonas aeruginosa*, H = *Haemophilus influenzae* type b., AX = *Alcaligenes xylosidans*, B = *Burkholderia cepacia*.
[b] Chronic colonization with PA according to antibody status (Mancini–Heremans Radial Immunodiffusion assay).

Table 2B
Patient characteristics and clinical markers at baseline and 5.5 months after baseline

Case #, gender, age (years)	Mutations	BMI (sex–age percentile)		Exacerbations (# in previous 6 months)		ALT (IU/L)	
		T_0	$T_{5.5}$	T_0	$T_{5.5}$	T_0	$T_{5.5}$
1, M, 8	DF508/DF508	14.1	14.6	2	0	22	24
2, F, 11	DF508/?	15.1	15.3	1	0	36	30
3, F, 19	DF508/G1244E	16.3	18.3	0	0	9	18
4, M, 5	DF508/R347P	14.7	15.4	0	1	11	11
5, M, 24	W1282G/G542X	17.8	19.7	1	0	16	20
6, F, 14	3659delC/?	16.6	19.3	0	0	22	22
7, F, 8	DF508/DF508	14.0	15.0	2	1	34	14
8, F, 7	Y1182X/G1244E	15.5	15.7	0	0	28	20
9, M, 27	DF508/2183delAA	18.1	20.0	3	1	48	31
10, M, 22	2183delAA/?	18.8	20.0	2	0	35	22
11, M, 15	DF508/R347H	17.5	18.7	1	1	10	18
12, M, 1	NA	NA	NA	NA	NA	NA	NA
13, F, 1	DF508/DF508	NA	NA	NA	NA	NA	NA

NA — data not available.
T_{-3} — 3 months prior to baseline; T_0 — baseline; $T_{5.5}$ — 5.5 months after baseline.

cases 4, 12, and 13, we can assess significance of change in clinical parameters over the 5.5 month period of initiation of the GSH regimen (Table 3).

5. Discussion

Though this observational study was uncontrolled, and therefore alternative explanations for the observed results are possible, the results are intriguing. Over a 5.5 month period, average lung function and weight parameters increased significantly, and number of bacteria cultures declined significantly.

From previous studies [18,22,23], we knew that inhaled buffered GSH would elevate blood and ELF levels of GSH and potentially increase lung function. These results corroborate previously published findings that improved lung function is associated with use of a GSH regimen in CF. Because these previous studies focused on lung function parameters, clinical indicators concerning the growth failure typical of pancreatic insufficient CF patients were not examined in detail. In fact, there was some reason to believe that oral glutathione would be of little use in the treatment of CF patients [7]. Nevertheless, these results show that significant weight gain is also associated with use of this GSH regimen. Given recent research findings showing a strong, significant, even predictive relationship between weight and lung function in CF [25–30], the significant weight gain observed in this 5.5 month study is of special note. While the U.S. Food and Drug Administration has recently asserted, "Literature reports clearly describe that orally administered glutathione is well absorbed" [24], the mechanism of oral uptake particularly requires further elucidation, especially in the case of CF patients whose glutathione system is abnormal.

The number of sputum cultures positive for all types of bacteria significantly declined over the study period. It is particularly interesting that 4 of the 5 cases that were originally positive for *Pseudomonas aeruginosa* (PA) were negative for PA 5.5 months later, including two of the three patients chronically infected with PA. Recent research has demonstrated that GSH in the ELF is specifically elevated through the CFTR channel in reaction to infection with *P. aeruginosa*, and assists in clearing that infection in normal organisms [4]. There was also a marked decrease in number of pulmonary exacerbations in this study, although a comparison is somewhat problematic given the slightly longer observation period at baseline (6 months prior to baseline versus 5.5 months study period). Additional research is required to determine if a GSH regimen

Table 3
Change in clinical markers over 5.5 months on a GSH regimen

Clinical marker	Baseline mean (SD)	Post-mean (SD)	Difference (post-base) mean (SD)	95% CI of difference	*p*-value
FEV1, % predicted ($N=10$)	54.2 (15.3)	60 (14.9)	5.8 (3.2)	(3.5, 8.1)	$p<0.0001$ [a]
Weight (%) ($N=13$)	10.9 (10.3)	19.5 (13.1)	8.6 (7.6)	(4.0, 13.2)	$p<0.001$ [a]
BMI (%) ($N=11$)	16.2 (1.7)	17.5 (2.2)	1.2 (0.8)	(0.7, 1.8)	$p<0.001$ [a]

Clinical marker	Baseline total #	Post-total #	Difference (post-base)	*p*-value
Positive sputum cultures ($N=11$)	13	5	6 declined 5 no change 0 increased	$p<0.03$ [b]
PA positive sputum cultures ($N=11$)	5	1	−4	No test done
Exacerbations ($N=11$)	12	4	−8	No test done

[a] Paired *t*-test.
[b] Sign test.

in CF patients helps normalize the immune response to PA infection in the consequences of CFTR dysfunction.

It should be noted that an FDA-approved oral formulation (Cachexon) is available in the United States, and two parenteral formulations (TAD, Ridutox) are approved in Italy. Also, GSH has been shown to be anti-genotoxic, anti-mutagenic, anti-carcinogenic, and anti-toxic with no known adverse side effects reported in the literature [31]. In this small observational study, we could detect no hepatotoxic effects, as measured by pre- and post-ALT levels.

This is not a call to encourage CF patients to begin treating themselves with GSH, but rather a call to researchers and clinicians to examine this therapeutic approach in a more rigorous fashion. Such an examination for inhaled GSH is already underway in Munich, Germany (PI: Matthias Griese), with a randomized, placebo-controlled trial of 24 week duration [32]. If a GSH regimen does ameliorate a number of important CF clinical markers, larger randomized, controlled studies of GSH augmentation in CF patients should be undertaken in as expeditious a manner as possible.

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