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Short Communication

Increasing sputum levels of gamma-glutamyltransferase may identify cystic fibrosis patients who do not benefit from inhaled glutathione☆

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Abstract

Glutathione (GSH) is decreased in cystic fibrosis (CF) airways, thus its resupply by inhalation has been employed to restore antioxidant defense. CF airways present however increased activity of gamma-glutamyltransferase (GGT), the enzyme specifically capable of degrading GSH, and thus inhaled GSH might be promptly catabolized. In addition, prooxidant reactions are known to originate during GGT-mediated GSH catabolism. We determined levels of GGT in the sputum samples obtained from a previously published trial of GSH inhalation treatment, and analyzed their correlations with inflammatory markers and FEV1% values. Results indicate that differentiating patients with increasing vs. decreasing GGT activity – as measured in sputum before and after the six months duration of the study – may discriminate subjects more likely profiting from inhaled GSH, as opposed to those with increasing GGT in which these treatments might even produce aggravation of the damage.

Keywords: Glutathione; Gamma-glutamyltransferase; Cystic fibrosis; Inflammation

Among major non-enzymatic antioxidants present in the airways – e.g. mucins [1] – reduced glutathione (GSH) is thought to represent a critical line of defense. GSH is sharply decreased in cystic fibrosis (CF) airways, mainly due to consumption during inflammation processes (reviewed in [2]). Thus GSH resupply by inhalation has been viewed as a direct tool to restore airways antioxidant defense. However, conclusive evidence that GSH inhalation indeed produces protective effects has never been provided in clinical studies [2].

One reason for this can be the increased activity of gamma-glutamyltransferase (GGT), the enzyme capable of degrading GSH. In CF airways increased levels are largely due to secretion of the enzyme by activated phagocytes [3]. GGT can rapidly degrade endogenous GSH as well as exogenous GSH administered by inhalation. Furthermore, it has been repeatedly shown that GGT-mediated degradation of GSH in the presence of transition metal ions can paradoxically exert prooxidant effects, through the formation of the highly reactive metabolite cysteinyl-glycine being able to start redox cycling reactions eventually leading to generation of reactive oxygen species and other free radicals [4–6]. Such GGT-mediated reactions can in fact modulate inflammation-related cellular components, such as cell surface receptors (e.g. TNFR-1) as well as transcription factors (e.g. NF-κB, AP-1), thus being potentially involved in modulation of the inflammatory response [4]. Against this background, differential levels of GGT in the airways might influence the final outcome of GSH inhalation therapies.

When correctly executed and of sufficient amount, sputum samples represent a reliable material for investigating pathological processes in respiratory diseases. Thus, in order to verify the

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hypothesis above, we determined GGT activities in the sputum samples obtained from a randomized, placebo-controlled trial previously published by our group [7].

In that study, inhaled GSH was evaluated in CF subjects with FEV1 values 40–90% of predicted. Subjects were randomized to receive glutathione (n. 73) or placebo (n. 80) every 12 h for 6 months. The study design consisted of a run-in period for determining baseline FEV1, and parallel treatment groups with assessments after 1, 3 and 6 months. The conclusions of the study were that GSH inhalation did neither produce significant change in markers of oxidation or inflammation, nor clinically relevant improvements in mean lung function.

We have now measured sputum GGT activities, and re-evaluated the clinical and biochemical responses in dependence of decreasing or increasing enzyme activities, as measured in sputum before and after the six months duration of the study. The following results were obtained:

a) *GGT activity.* GGT levels that were detectable in the solubilized sputum supernatants at baseline ranged from 0.12 to 213.07 mU/ml (n = 54; median 23.80). At the end of treatment, pre–post differences of GGT activities (delta's, dGGT) did not differ significantly between placebo and GSH-treated group. As hypothesized [3], a strong correlation was observed between GGT activity and sputum neutrophils count as well as neutrophil elastase (Spearman: r = 0.66 and 0.68, respectively; $p < 0.0001$).

b) *Sputum GGT and low molecular thiols (LMWT).* Sputum GGT levels at baseline showed significant correlations with total levels (i.e., reduced plus oxidized) of all low molecular thiols (LMWT) measured: cysteine, cysteinyl-glycine, glutathione, gamma-glutamylcysteine and homocysteine (Spearman r values in the range 0.49–0.68), suggesting a role of airways GGT in metabolism of GSH.
Increases and decreases in GGT activities during the study (dGGT > 0 and dGGT < 0 values) were consistently associated with increases and decreases, respectively, of all LMWT in both placebo and treated patients. Importantly, total levels of cysteinyl-glycine (i.e. reduced plus oxidized) were much more increased in GSH-treated/GGT-increased patients (median values: +56.1 vs. +9.3 pM, $p < 0.03$). Since cysteinyl-glycine is the metabolite specifically originating from GGT-mediated metabolism of GSH, this data suggests that inhaled GSH was indeed undergoing GGT-mediated metabolism in the patients' airways.

c) *Protein oxidation.* In the previous study [7] carbonylated proteins were determined as an oxidative stress index, but mean values were not significantly different in GSH vs. placebo group. Interestingly, our re-analysis showed that GSH-treated patients with increased sputum GGT had significantly higher pre–post differences in levels of protein carbonyls at the end of GSH treatment, as compared to the corresponding placebo group (Table 1).

d) *Inflammatory cytokines.* Sputum levels of proinflammatory cytokines IL-8, TNF-α and IL-1β were also evaluated. No significant pre–post differences between placebo and GSH-treated group had been observed in the previous study [7].

Table 1
Changes with treatment (pre–post differences: delta's) of protein carbonyls, inflammatory cytokines and other parameters in sputum of patients with increasing vs. decreasing sputum gamma-glutamyltransferase activity (GGT) levels.

	Placebo				GSH				
	A. Decreasing sputum GGT activity, dGGT < 0		B. Increasing sputum GGT activity, dGGT > 0		C. Decreasing sputum GGT activity, dGGT < 0		D. Increasing sputum GGT activity, dGGT > 0		
Delta's	N	Medians (25th; 75th percentile)	N	Medians (25th; 75th percentile)	N	Medians (25th; 75th percentile)	N	Medians (25th; 75th percentile)	p-Value
Protein carbonyls [U]	8	0.9 (−0.7; 9.3)	11	−4.7 (−12.6; 6.7)	10	−3.2 (−24.3; 0.7)	9	**5.2 * (0.3; 41.3)**	*p < 0.05 as compared to B and C
IL-8 [pg/ml]	11	105.9 (−439.1; 780.3)	10	254 (75.8; 1127)	10	**−947.5* (−3852; −379)**	8	986.3 (172; 2169)	*p < 0.05 as compared to B and D
TNF-alpha [pg/ml]	11	0.2 (−4.7; 4.5)	13	2.7 (−3.5; 23.6)	10	**−5.9 * (−129.1; −1.4)**	6	82.7 (−24.9; 371.4)	*p < 0.05 as compared to B and D
IL-1β [pg/ml]	12	−4.6 (−705.1; 8.4)	12	20.4 (−0.8; 129.4)	10	**−660.5 * (−1813; −43.5)**	8	63.53 (3.4; 448.7)	*p < 0.05 as compared to B and D
Neutro-phil elastase [μg/ml]	8	**−56.7 * (−264.1; −14.6)**	11	56.9 (−20.8; 111.8)	10	**−74.2 * (−358; 7.2)**	6	28.2 (13.9; 183.2)	*p < 0.05 as compared to B and D
FEV1%	9	3.0 (−5.5; 7.5)	12	−5.0 (−8.7; 0.5)	10	**6.0 * (2.0; 9.3)**	8	−2.5 (−7.5; 2.7)	*p < 0.05 as compared with B and D

p-Values were obtained by Kruskal–Wallis test with Dunn's correction for multiple comparisons.